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A CASE OF PYELONEPHRITIS OF PREGNANCY DUE TO EBERTHELLA ALKALESCENS*†

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THE patient, a married woman, twenty-three years old, primipara, was admitted to the hospital on December 18, 1928, with a history of a three months' pregnancy complicated by pain in the lower abdomen, nocturia, dysuria, and pyuria. This condition was aggravated by nausea, vomiting, and high temperature of the remittent type, all of which symptoms began three weeks previous to admission to the hospital. No pathologic condition of special significance was noted in the past history, with the exception of jaundice eleven years before this illness. On the basis of the physical examination and symptomatology the case was diagnosed as pyelitis complicated by the vomiting of pregnancy.

Repeated cystographic examinations showed generalized cystitis with both ureters in normal position and with no compensatory dilatation or angulation. The urine from both kidneys was full of pus. The functional phenolphthalein test after fifteen minutes gave 25 per cent on the left side and 0 on the right side. X-ray pyelographic examination showed normal position of the kidneys with no pathologic condition in the kidney pelvis and ureters.

On laboratory examination catheterized urine from both kidneys was found to contain pus and a large number of gram-negative bacilli resembling B. dysenteriae. Repeated examination of the stool revealed the presence of an organism similar to that found in the urine. Other laboratory findings of note were a negative blood culture, a rather high icteric index (20), positive

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occult blood in the stool, secondary anemia with 60 per cent hemoglobin and 3,200,000 red blood cells, and a negative Wassermann. In view of the progressing toxemia, vomiting, and failure of conservative treatment, including antiseptic lavages of the kidney pelvis, a therapeutic abortion was advised and done eighteen days after admission with the most satisfactory result of an uneventful and complete recovery.

The unusual bacteriologic findings of the same organism in both urine and feces stimulated more detailed studies which yielded the following results: The organism is a gram-negative bacillus, rather short and sometimes plump; nonmotile; does not produce indol or reduce nitrates and does not liquefy gelatine. It grows well on plain agar with small grayish colonies. On Russell's double sugar media it produces an acid butt, alkaline slant, and no gas. Litmus milk is slightly acidified at the beginning but no coagulation occurs. This is followed immediately by alkalinity which gradually progresses. There is slight discoloration of lead acetate agar. On eosin methylene blue it forms small grayish white colonies. The fermentation reactions are presented in Table I.

TABLE I

FERMENTATION REACTIONS

CARBOHYDRATE	PATIENT'S STRAIN	B. ALKALESCENS AMER. TYPE. COL.	B. DYSENTERIAE FLEXNER	B. JEFFERSONII R. I. EXP. STA.
Dextrose	A	A	A	A
Levulose	A	A	A	A
Galactose	A	A	A	A
Xylose	A	A	O	slow A
Rhamnose	slow A	slow A	O or A	slow A
Maltose	A	A	A	A
Lactose	O	O	O	O
Saccharose	A or O	O	O	A
Raffinose	O	O	A or O	O
Dextrin	A-alk.	O	O	A-alk.
Inulin	O	O	O	O
Mannite	A	A	A	A
Dulcite	A	A	O	A
Salycin	O	O	O	O

A—indicates production of acid; no gas.
O—indicates no reaction.

It is evident from Table I that the isolated organisms differ essentially in their fermentation reactions from:

1. B. dysenteriae Flexner in the fermentation of xylose, dulcite, and rhamnose.
2. Eberthella alkalescens in the fermentation of dextrin.
3. Eberthella jeffersonii only in the fermentation of xylose, which was originally described as fermenting xylose.

AGGLUTINATION STUDIES

Agglutination studies were made (1) using the organism isolated from the patient and known antisera; and (2) known organisms and the patient's serum:

1. Organism isolated from patient and known antisera.

TABLE II

	ANTISERA	RESULTS
Organism from patient	Para A and B, B. typhosus antiserum	No agglutination
	B. dys. Mt. Desert "	No agglutination
	B. dys. Shiga "	No agglutination
	B. dys. Flexner "	1–200
	Polyvalent dys. "	1–200
After absorbing with patient's organism and retested with B. dys. Flexner. (After method of Castellani.)	B. dys. Flexner antiserum	1–200

It will be seen from Table II that:

(a) There is no agglutination in Para A, Para B, or B. typhosus antisera.

(b) There is no agglutination in B. dysenteriae Mt. Desert antiserum.

(c) There is no agglutination in B. dysenteriae Shiga antiserum.

(d) All strains when first isolated agglutinated partially in 1:200 dilution with polyvalent dysentery antiserum.

(e) All strains were partially agglutinated in 1:200 dilution by B. dysenteriae Flexner antiserum.

(f) After absorbing with the unknown organism the B. dysenteriae Flexner antiserum still agglutinated B. dysenteriae Flexner, showing that the organism was not identical with B. dysenteriae Flexner, although very closely related.

2. Patient's serum and known organisms.

TABLE III

	ORGANISM	RESULTS
Patient's serum	Patient's organism	1–640
	Eberthella alkalescens	1–640
	B. dys. Flexner	1–100
	B. dys. Shiga	0
	Para A and B, B. typhosus	0
After absorbing patient's serum with B. dys. Flexner, and retested with the patient's organism	B. dys. Flexner	1–640

It will be noticed in Table III that:

(a) The patient's serum and the organism isolated from the urine and stool agglutinated up to 1:640 dilution. This occurred upon two different examinations during her stay in the hospital. This differs from the case reported by Andrewes (Lancet **1**: 560, 1918) as in no case did he find an agglutination between the organism isolated and the patient's serum.

(b) There is no agglutination with Para A, Para B, or B. typhosus.

(c) There is no agglutination with B. dysenteriae Shiga.

(d) There is partial agglutination up to 1:100 dilution with B. dysenteriae Flexner.

(e) After absorbing the patient's serum with B. dysenteriae Flexner, its ability to agglutinate the organism isolated is still retained, showing that while closely related the organisms were not identical.

(f) The patient's serum and Eberthella alkalescens agglutinated up to 1:640 dilution.

Unfortunately the culture of Eberthella jeffersonii, which it also resembles very closely culturally, was obtained too late to make any agglutination studies with it.

DISCUSSION

This description indicates that the organism isolated belongs to the genus Eberthella (S. A. B.). It shows a very close relationship to Eberthella paradysenteriae (Flexner) but is not identical with it, as is shown by the carbohydrate reactions (Table I) and its agglutination with Flexner antiserum (Table II). It bears a much closer relationship to Eberthella alkalescens (Andrewes), differing in its fermentation of dextrin and in the fact that it does not produce indol. However, the agglutinations were complete in the case of the patient's serum and Eberthella alkalescens. Culturally, it is almost identical with Eberthella jeffersonii, but as already mentioned no agglutination tests were made with this organism. It may be assumed, therefore, that the organism is either B. alkalescens or B. jeffersonii, the preponderance of data identifying it with Eberthella alkalescens.

The fermentation characters and agglutination features of this nonmotile, gram-negative bacillus found both in the urine and feces correspond to a nonlactose-fermenting bacillus described by Andrewes (1918) and named by him B. alkalescens. The relation of this organism to human pathology, and especially to urinary pathology, is not sufficiently studied on account of the rarity with which this organism is met in clinical work. Recently J. Smith and A. Fraser of England (1928) described a case of continuous fever due to B. alkalescens. This case is of special interest since it was observed in a young woman who developed three days after delivery marked toxemia and pyrexia of remittent type which lasted for nineteen days. Blood culture, feces, urine, and material from the uterus all contained the same nonmotile, gram-negative, nonlactose-fermenting bacillus. While the urinary symptoms were absent, being manifested only by transient albuminuria, there were present, as in our case, generalized icterus, abdominal distention, persistent hiccup, loose stools, and secondary anemia with 2,020,000 red blood cells.

The studies on pathogenicity of this organism made by these authors showed that guinea pigs and mice are rather sensitive to this organism and readily die after inoculaton, while the rabbits remain unaffected. The close agglutination reaction of this organism with the Flexner dysenteriae group involves immediately the general question of dysenteric bacteriemia and dysenteric pyelonephritis. Kostrzewski (1921) found that in the Shiga-Kruse dysenteriae blood cultures were positive in 38.69 per cent and the positive cultures were most often observed between the third and ninth days of the disease.

Cases of dysenteric pyelonephritis are extremely rare. Calalb and Ionesco (1925) reported the case of a primipara in the eighth month of pregnancy with bilateral purulent pyelonephritis which on bacteriologic examination showed a pure culture of nonmotile, gram-negative bacilli with all the characteristics of Flexner's organism. Out of eighty-two cases of urinary infection, Hilgers of Germany (1919), isolated pseudodysenteric bacilli only twice. One of these cases at the beginning was agglutinated only by anti-Shiga serum but later was agglutinated also by anti-Flexner serum. Of course, in interpreting the agglutination of dysenteriae bacilli by the serum of a pregnant patient (especially at the end of pregnancy), one should keep in mind the observation of Lowenthal of Switzerland (1920) who showed that a good 38 per cent of pregnant patients give a positive agglutination test with Flexner's Y strain. This phenomenon, as was shown later, does not depend upon a change in blood lecithin or blood cholesterol and evidently is caused by unknown biophysic changes of serum during pregnancy.

Regarding the source and routes of infection in our particular patient in whom right side involvement was predominant, in whom feces and urine both contained the same organism, and in whom the therapeutic abortion led to complete and speedy recovery, it is more reasonable to think of lymphogenous infection from the large bowel with constipation as a coexisting and predisposing factor.

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CLINICAL TREATMENT OF ASTHMATIC PATIENTS IN ALLERGEN PROOF CHAMBERS*

(Report Founded on 1000 Cases)

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SINCE the installation of allergen proof chambers in our clinic for allergic diseases five years ago, we have treated one thousand asthmatic patients with this method. The starting point of this new way of treatment was the consideration that good results observed in high-mountain air could be obtained also in lower regions, since the beneficial influence of high altitudes on asthma cases is almost entirely due to the absence of house allergens. We have proved that even in our lowlands patients could be kept in an allergen free environment during the night and part of the day, by relatively simple technical methods.

After observing the first eighty patients in our new clinic, Kremer and myself stated that our results were similar to those obtained at high altitudes, i.e., 70 per cent were free from asthmatic symptoms after some days, 20 per cent were much improved after two or three weeks, and 10 per cent remained refractory to the treatment.

Our results with the first 1000 patients are still about the same, but our later experience has enabled us to offer some additional remarks on the subject.

During the first period of our work with allergen proof chambers, we were as a rule unable to predict with any certainty whether or not a given patient would benefit by residence in the chamber. We now have found however that often a more definite prognosis may be given.

Many years ago our attention was drawn to the fact, that many allergic patients are hypersensitive to drugs of the coal tar series, but our observations in this respect had only been occasional ones.

During later years we have studied the matter more systematically, and made it a routine to test every asthmatic patient that came under our treatment for his sensitivity to coal tar products, taking aspirin as test object. As a result of this study, which has been published extensively elsewhere† we found that not less than 10 per cent of our asthmatic patients are hypersensitive to these drugs. We furthermore found that these cases of aspirin hypersensitiveness form a separate group, indicated by us as the "aspirin group," which has definite characteristics, one of them being that the patients are rarely or almost never benefited by residence in the allergen proof chamber.

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†W. Storm van Leeuwen, Pathognomonische Bedeutung der Uberempfindlichkeit gegen Aspirin. München. med. Wchnschr., No. 37, 1588, 1928.

Another characteristic is that the routine methods of treatment, including specific desensitization, for the most part give poor results in these cases so that they have to be treated with nonspecific methods.

Every clinician therefore who makes observations in the allergen proof chamber, should bear in mind that the "aspirin group" is unfit for this kind of treatment.

Another point of interest is connected with the social state and with the original residence of the asthmatic patient.

We have repeatedly drawn attention to the fact, that the conditions of climate and soil are of prime importance for the well-being of the asthmatic patient. A moist soil (clay and peat for example) with high level of groundwater and high water-absorbing power, is as a rule bad for asthmatic patients, whereas a dry soil (sand, chalk, rock, Kieselguhr) is good. We have shown that in some parts of our country the percentage of the whole population who suffer from asthma is extremely high, so that asthmatic patients living in these regions belong almost entirely to the climate group, whereas the percentage of climate cases in other regions is lower. Tiefensee, who has recently made an extensive statistical study of more than 1100 cases of asthma in East Prussia, came to practically the same conclusion.

Now it is evident that the condition of the soil will have much more influence on people living in badly constructed, small houses than on people who have been able to build their homes in the best situations with large foundations, perfect material, central heating, etc., and consequently the probability that an asthmatic patient, coming from a certain region, will react favorably to the allergen proof chamber is greater if the conditions in his own house are bad. Hence wealthy people living in dry places will have less chance of becoming climate asthmatic patients than people from the laboring or poorer classes who are living in moist regions, and the percentage of cases reacting favorably to residence in the allergen proof chamber will be much lower in the first group than in the second. Wealthy persons from Berlin (built on Kieselguhr) or London (built on chalk) will perhaps have a 40 per cent chance of belonging to the climate group, whereas persons from the labor classes in Rotterdam will show a percentage as high as ninety.

Clinicians who are going to start this kind of work will meet with a great many successes if their clinical material is drawn from laborers or paupers from moist regions, but they will have much less success with wealthy individuals, especially with those living in places where there is little asthma. The author's experience is that if a very wealthy man becomes asthmatic, he belongs for the most part to the bronchitis group, or to the animal dandruff or foodstuff group.

From the beginning of our work with allergic diseases the author has realized that the aim of this kind of treatment should not be to obtain a *cure* for the patient, simply by inducing him to sleep for some weeks or even longer in the chamber. Experience has taught all of us that residence in high altitudes very rarely relieves the patient of his illness permanently; it only gives temporary relief by eliminating the allergens. Since we know that the most effective elimination of the specific allergens such as occurs during autumn

and winter in hay fever cases, almost never *cures* the illness, we could predict that neither high altitude nor the allergen proof chamber could, per se, produce a cure of the asthmatic condition. As has been said, experience has taught us the correctness of this conception, but it also has taught us that it is extremely difficult to induce our patients (especially the more wealthy ones), or even to induce the physicians, to accept this condition. The patient wants to obtain a *cure* in the same way that he hopes to be cured of his rheumatism by residence in a "Kurort" or spa.

The real objects of the admission of asthmatic patients to the allergen proof chamber in the clinic are: (1) *Observation*, which enables us to make a correct diagnosis of the case within a few days or at most within two weeks, so that the pure climate cases may be differentiated from the foodstuff cases, from the bronchitis cases, from the aspirin group and from the mixed cases. (2) *Temporary improvement* which enables us to judge the prognosis and the efficiency of the patient for work (vide our article on functional emphysema). (3) The *possibility of desensitizing* the patient to all the allergens to which he reacts in a short time and in a very efficient way; also in cases in which desensitization at home would only be possible with great difficulty or even danger, on account of the fact that at home large, unknown and varying quantities of allergens may act on the patient. We often give therapeutic allergen injections two or three times daily in increasing doses to our patients during residence in the allergen proof chamber, even with extract of housedust which in our hands has often proved to be dangerous in ambulatory patients. This residence in the allergen proof chamber gives the patient a complete relief from attacks so that he may be induced to have an allergen proof installation in his own house. This is the most efficient treatment of climate cases of allergy and is based on the most important factor, elimination of the allergens.

More than 60 allergen proof installations have now been made in private houses. In the beginning the results were, owing to technical mistakes, not altogether satisfactory. With the last twenty-five installations however, which were technically correct, we have had 22 very good results, 2 moderate results, one complete failure.

In our discussion of the influence of the allergen proof chamber on the number and intensity of the asthmatic attacks we have frequently used the terms "improvement," "relief from attacks," etc. We are able however to express these influences on the patient's condition in a more objective way, by taking his respiration-curve. These observations, which have given us very satisfactory results during the last years, have been discussed in detail in a paper on functional emphysema.

For the present purpose it is sufficient to state that we take our respiration-curves with a simple spirometer, which writes its excursions directly on the moving surface of a kymograph. The velocity of this kymograph has been so chosen that 2 mm. corresponds to one second, and the spirometer is so adjusted that an excursion of 1 cm. corresponds to about 450 c.c. of air. The spirometer is filled with oxygen. The patient breathes and rebreathes in the spirometer, but the time of the experiment is so short that there is no considerable accumulation of CO_2. We always ask the patient first to make some

ordinary respirations, then we induce him to make an inspiration as deeply as possible, and then as quickly as possible make the maximum expiration. The time between the beginning of the deep inspiration and the end of the deep expiration is called respiration time, whereas the difference between the lowest point of the inspiration and the highest point of the expiration gives the vital capacity. We found in work done with Mr. Storm* that the vital capacity does not give a good indication of the condition of a patient suffering from asthma or functional emphysema, whereas the respiration time gives a very good indication of his condition.

Fig. 1 is the respiration curve of a man sixty-five years old, who had suffered from asthma for sixty years. His respiration curve indicated a considerable degree of functional emphysema, although the vital capacity was still 3000 c.c. He was a complete invalid at the time the curve was taken, and his respiration time was fifteen seconds (normal about five or six seconds).

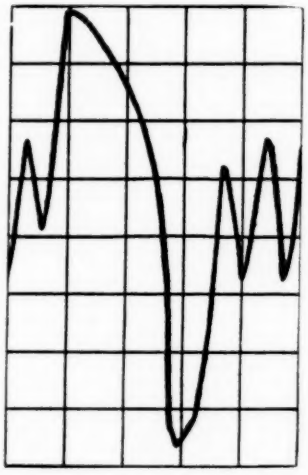

Fig. 1.—Functional emphysema in patient suffering from asthma for 60 years. Vital capacity 3000 c.c. Respiration time fifteen seconds.

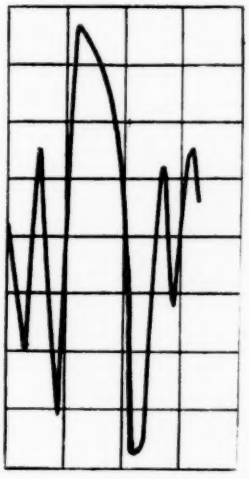

Fig. 2.—Functional emphysema, same case as Fig. 1, after four days in allergen proof chamber. Vital capacity 3000 c.c. (unchanged). Respiration time eight seconds (reduced to about one-half).

After four days' residence in the allergen proof chamber, the patient was almost completely relieved of asthmatic attacks, he felt quite well, could walk, go up and down the staircase, etc., and his respiration curve showed a considerable improvement (Fig. 2). The respiration time was reduced to eight seconds. The vital capacity remained about the same.

Further details of this very simple device for getting an impression of the efficiency of the asthmatic or sufferer from functional emphysema is to be found in the paper cited above.

Hitherto the influence of the allergen proof chamber on asthmatic patients has alone been mentioned. It should, however, not be forgotten that the possibilities of treatment with this chamber go far beyond the limit of

*Storm van Leeuwen, W. and Storm, C. J.: Ueber der Begriff des functionellen Emphysems und seinen Wert für Diagnose und Prognose beim Asthmatiker. München. med. Wchnschr. Nos. 10 and 11, pp. 401 and 457, 1929.

these allergic diseases. Incidentally it may be remarked that cases of whooping cough cleared up extremely quickly in our allergen proof chamber ventilated with purified air, but the greatest importance must be attached to the fact, that the chamber has proved to be of great benefit for cases of tuberculosis.

In the beginning of our work on asthma in connection with house-allergens, we sometimes had to treat patients suffering from asthma and tuberculosis, and we were surprised to find how quickly and efficiently those patients who suffered from a combination of these diseases were cured by residence in the chamber. Not only did they get very quick relief from their asthmatic symptoms, but also the tuberculous process improved considerably. This induced us to try the effect of the chamber on cases of tuberculosis without asthma. We have now treated 35 patients with tuberculosis in the allergen proof chamber. Although this number is limited, the results in the appropriate cases were so striking that I feel justified in stating that the allergen proof chamber enables us to treat cases of tuberculosis in any place and in all climates with at least as good results, as high altitudes would give in these cases.

In the chamber the tuberculous patient breathes air free from house-allergens, and which, by purification and refrigeration, has become absolutely pure and very dry. In the exceptional cases, in which the patient does not stand very well the breathing of very dry air, it is of course easy enough to moisten this air. Anyhow this installation enables us to have the patient during weeks and months in a constant environment with regard to air purity and humidity. If ultraviolet light is desired, this of course may be very easily obtained by using a quartz-lamp and in this way the chief beneficial effects of high altitude on tuberculosis are available, as stated above, at any place and in any climate, and in a constant form during the whole year.

High altitude of course means lack of oxygen. Some authors believe that breathing air which is deficient in oxygen might be beneficial for the tuberculous patient. Of course it is very difficult to prove the inaccuracy of such a statement, but the author draws attention to the following two points.

1. It is highly improbable that a sickness would be benefited by lack of the principal foodstuff, i.e., oxygen.

2. We all know, that one of the first principles in the treatment of any sick organ, and especially of the lungs, is keeping the organ at rest. Lack of oxygen in the air however must involve larger movements of the chest and of the lungs, and so it is very difficult for the author to accept the theory that lack of oxygen might be beneficial for the patient.

Moreover it is known, that in the severest cases of tuberculosis the patients are not allowed to go to high altitudes. As experience has taught they do not stand the climate. Such a contraindication would never exist for treatment in the allergen proof chamber. The breathing of purified air can never be a disadvantage. Breathing of too dry air might be a disadvantage, but as stated above, this is very easily avoided by moistening the air.

With regard to the final results of treatment of tuberculous patients in the allergen proof chamber, it must of course be stated from the beginning

that severe cases with large cavities, high temperature, etc., may improve at the beginning, but will not be cured.

We have studied the influence of the chamber on a number of such cases and found that, although no harm was ever done and although improvement could be seen at first these patients were not cured, as of course could have been anticipated. But on the other hand we also found that the more suitable cases did extremely well even if the temperature was rather high and though the sputum contained a great many tubercle bacilli, and small cavities were present.

STUDIES ON THE TOXICITY OF ALPHA-BUTYLOXY-CINCHONINIC ACID DIETHYL-ETHYLENE-DIAMIDE HYDROCHLORIDE (NUPERCAINE)*

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CONSIDERABLE interest has been evinced in a new local anesthetic; alpha-butyloxy-cinchoninic acid diethyl-ethylene-diamide hydrochloride, which is marketed abroad under the name percain and in America as nupercaine. For the sake of simplicity, the latter designation will be used in the present paper.

According to most of the already numerous clinical reports, this compound is an effective anesthetic either on topical application to mucous membranes or after injection.

The toxicity of any therapeutic agent is, obviously, a matter of great practical importance. From the investigations on laboratory animals reported by Uhlmann,[3] Lipschitz and Laubender,[2] and Gessner and Nauheimer,[1] it is obvious that nupercaine is extremely poisonous; its toxicity being variously placed at from twice to five times that of cocaine. To compensate for this startlingly high toxicity, nupercaine is stated to be a very powerfully acting anesthetic; so powerful that the claim has been made that in the high dilutions which are found effective, it is actually safer than the customarily used local anesthetics.

Preliminary investigations carried out in this laboratory with the object of studying the toxicity of nupercaine, yielded results somewhat at variance with those reported by Uhlmann[3] and by Lipschitz and Laubender.[2] This led to a more detailed study, which forms the basis for the present report.

Uhlmann[3] states that the M. L. D. of nupercaine for guinea pigs is 7.5 mg. per kg. and for rabbits is 10 mg. per kg. when injections are made subcutaneously, but that the dog survives subcutaneous doses "slightly larger" than those fatal for the rabbit.

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The lethal dose determined by us for guinea pigs and dogs following the subcutaneous injection of a 1 per cent solution of nupercaine in saline during the latter part of December, 1929, differs quite markedly from that reported by Uhlmann.[3] Thus guinea pigs survived the injection of 10; 15; 17.5 (2 animals); 18; and 19 (2 animals) mg. per kg. respectively; one of three animals succumbed to a dose of 19.5 mg. per kg.; and both pigs that received 20 mg. per kg. died within twenty-four hours. It is obvious, therefore, that under the conditions of our experiments, the resistance of guinea pigs to subcutaneously injected nupercaine is more than two and a half times that found by Uhlmann.

Uhlmann is quite indefinite regarding the M. D. L. of nupercaine subcutaneously administered to dogs; one gets the impression that he considers it close to 10 mg. per kg. None of three dogs injected by us in December, 1929, with 20 mg. of nupercaine per kg. in the form of 1 per cent solution in saline died; one of two animals receiving a subcutaneous injecton of 21 mg. per kg. died in thirteen minutes; a similar result being obtained on increasing the dose to 22 mg. per kg. Three out of five dogs receiving 25 mg. per kg. succumbed and death was produced in all five cases where the animals were given 25 mg. of nupercaine per kg.

Several explanations may be suggested to account for this discrepancy between our results and those of Uhlmann. It is possible that this latter investigator used a solution of nupercaine more concentrated than 1 per cent; or that with guinea pigs his injections were inadvertently made into the peritoneal cavity; finally, one must take into consideration the possibility of seasonal variation in the resistance of the animals.

Injected intravenously into dogs, the M. L. D. of nupercaine was found much lower than when the drug was injected subcutaneously. Doses of 1.5; 2.0 (2 animals); and 2.5 mg. per kg. (2 animals) did not cause death when rapidly injected into the marginal ear vein; all five animals receiving 3 mg. per kg. in this manner succumbed. In these experiments, as with subcutaneous injection, a 1 per cent solution of nupercaine in 0.9 per cent sodium chloride was used.

Nupercaine is readily absorbed from the nasal mucosa as shown by experiments carried out on dogs. The fatal dose by this mode of administration is approximately five times as large as by the intravenous route. Varying amounts of a 10 per cent solution of nupercaine were instilled in the nares of unanesthetized dogs by means of a pipette, the heads being held in an upright position. The total amount of soluton injected in each case was so small that it was found unnecessary to plug the nares with vaselined cotton to prevent leaking. The application of 15 mg. of the product per kg. to the nasal mucosa of dogs is fatal within twenty minutes. Smaller amounts, while producing alarming symptoms, are tolerated and the animal recovers almost completely within a few hours. Below 5 mg. per kg., animals show but slight effects from the application.

The rapidity of absorption of nupercaine from the nasal mucosa is shown by the fact that after the application of from 5 to 10 mg. of the drug per kg. in a manner as described above, tremors, excitement, spasticity of limbs, eme-

sis, and even convulsions are produced within a few minutes. The severity of the symptoms occurring within this time from such a dose is roughly comparable to that following the intravenous administration of one-third of a minimal fatal dose of the preparation.

It should be borne in mind, however, that we are employing many times the dose and concentration of the drug that would ever be used clinically. Combinations of nupercaine and epinephrine are recommended on the basis that the latter, by its vasoconstrictor action, lessens materially the absorption of the anesthetic. The fact should be emphasized that absorption of nupercaine through the nasal mucosa occurs with such rapidity that epinephrine is of little value in restraining the entrance of the anesthetic into the circulation, although it is quite effective in retarding absorption after subcutaneous injection. This statement was attested by experimental evidence in which it was observed that the simultaneous administration of epinephrine did not lower the toxicity of nupercaine when applied to the nasal mucosa. A more desirable procedure would seem to consist of a preliminary application of epinephrine followed by the anesthetic. Effects similar to those following application of nupercaine to the nasal mucosa, are observed also when the compound is applied to the buccal or pharyngeal mucous membrane.

Absorption of nupercaine from the stomach is slow and uncertain. Animals, starved for twenty-four hours, and receiving varying doses of nupercaine by stomach tube, showed that the compound possesses a relatively low toxicity by this mode of administration. The minimal lethal dose by stomach is estimated at approximately 80 mg. per kg. Dogs survive 50 mg. as a general rule, although in one instance this dose proved fatal. In unmorphinized animals emesis occurred with such regularity that satisfactory results were not obtainable. The subcutaneous administration of 5 mg. of morphine sulphate per kg. was sufficient to prevent the emesis in every case. Doses of nupercaine ranging from 50 to 100 mg. per kg., were given to these depressed animals through a stomach tube. One animal receiving 50 mg. died within a few minutes, another receiving the same dose recovered completely after exhibiting the characteristic symptoms of nupercaine poisoning. One dose of 75 mg. per kg. was survived, while doses above this amount were invariably fatal. As a matter of general interest it might be pointed out that the emetic action of nupercaine is central in origin, resulting from direct stimulation of the vomiting center, and is one of the earliest indications of the toxic action of the anesthetic.

Absorption of nupercaine from the bladder does not seem to occur to any appreciable extent, or else it takes place so slowly, and destruction of the compound in the body is so effective, that a toxic amount of the anesthetic does not accumulate in the blood stream. Dogs anesthetized by the intraperitoneal injection of chloretone, and arranged for recording blood pressure and respiration, gave no evidence of systemic reaction to the instillation of 30 mg. nupercaine per kg. into the previously irrigated and evacuated bladder, observatons being made during the course of three hours.

Since the compound is recommended for gynecologic surgery, it was deemed of practical importance to determine the absorbability of nupercaine

from the vaginal canal. In our first experiments, 20 mg. per kg. in 10 per cent solution was injected into the vaginal canal by means of a soft rubber catheter connected with a syringe. The orifice was then closed by means of a pledget of vaselined cotton, and the animal observed throughout the day. There was not the slightest evidence of absorption as could be ascertained from the animals behavior. Feeling that animals can tolerate this dose with impunity, larger amounts up to 100 mg. per kg. were employed. In these latter amounts it became necessary to insert the pure crystals of nupercaine against the cervix by means of a small speculum. Here again the compound seemed to be perfectly innocuous. It seems, therefore, that nupercaine is very poorly absorbed from the vaginal mucosa, certainly at a rate much slower than the rate of destruction of the compound in the body. That local anesthesia was effected was clearly shown by the failure of the animal to respond to mechanical stimulation of vaginal mucosa with needles, and by pinching with a hemostat.

Lipschitz and Laubender[2] state that nupercaine once in the circulation of rabbits is slowly eliminated. Their observations are quite at variance with the results obtained in this laboratory on the elimination and detoxication of the compound by cats and dogs. The fact that the subcutaneous fatal dose of nupercaine is approximately eight times the intravenous dose, whereas with cocaine the ratio of subcutaneous and intravenous toxicity is 2:1, might be taken to indicate that either nupercaine is four times as rapidly eliminated as cocaine, or else cocaine is four times as rapidly absorbed. That the latter does not seem to be the case is shown by the observation that animals recover much more promptly from sublethal doses of nupercaine than in the case of cocaine. Experiments have been conducted in this laboratory in which dogs received one-half of the minimal fatal dose of nupercaine intravenously at ten minute intervals. Each injection was followed immediately by the characteristic tremors, convulsions, and dyspnea but these symptoms subsided rapidly. By dividing the fatal dose of nupercaine in this manner it was shown that dogs would tolerate a quantity of the anesthetic equivalent to two or three times the fatal dose, administered within an hour. Experiments of this nature have been carried out repeatedly on both dogs and cats with similar results. Animals receiving sublethal doses at ten minute intervals will tolerate several times the minimal fatal dose by rapid intravenous injection. It seems quite evident, therefore, that nupercaine is rapidly eliminated or detoxicated by the body. The mechanism of elimination of the compound has not been sufficiently investigated to justify a report at this time. It is believed that the liver plays an important part in the destruction of nupercaine.

SUMMARY

The minimal fatal dose of nupercaine is approximately 20 mg. per kg. when injected subcutaneously into guinea pigs, and 25 mg. when similarly injected into dogs. The intravenous fatal dose for dogs is determined to be between 2.5 and 3 mg. per kg.; animals reacting uniformly to toxic amounts of the drug.

Experiments carried out in this laboratory show that nupercaine is rapidly absorbed from the nasal and buccal mucosae, but very poorly from the bladder or vaginal canal.

Contrary to previous existing reports, nupercaine seems to be quite rapidly detoxicated in vivo.

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THE RÔLE OF SERUM GLOBULINS IN THE WASSERMANN REACTION*

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SINCE the application by A. von Wassermann in 1906 of the principle of complement fixation, previously described by Bordet of the Pasteur Institute, to the serum diagnosis of syphilis, numerous attempts have been made to improve upon the technic of the test. Some of these have been very successful in producing a more reliable technic while others were less so.

Noguchi[1] in 1909 published "A New and Simple Method for the Serum Diagnosis of Syphilis" in which he employed the antihuman hemolytic system and used much less quantities of reagents than Wassermann used. His method of adjusting the hemolytic system remained similar to the original in that he used a fixed amount of complement serum with two units of titrated amboceptor. About the same time McKenzie,[2] and Browning and McKenzie[3] devised a method of measuring the anticomplementary activity, in units, of serum and antigen alone and of normal serum and antigen together, and deducting that amount from the total number of units absorbed by the extract in the presence of syphilitic serum.

Thompson[4] in 1914 conceived the idea of titrating the complement serum. He used the antihuman hemolytic system and added antigen and a small amount of normal human serum to the tubes of his titration in order to allow for any anticomplementary activity of the serum or antigen alone. This seems to have been a big step in the improvement of the test for syphilis.

In 1916 Thomas and Ivy[5] employed a similar technic to Thompson's but using an antisheep hemolytic system. They reported that a larger dose of complement was indicated when the normal serum was added to the titration than if it were left out.

Another vote for the titration of complement instead of using a fixed amount with a titrated amboceptor was cast by Ottenberg[6] in 1917 when,

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after extensive investigation relative to the hemolytic system, he concluded: "The correct method of compensating for complements of greater than average hemolytic strength is by using a smaller dose of complement."

The technic recommended by Kolmer and Boerner,[7] after a very thorough and extensive study of factors influencing the Wassermann reaction, probably is the most noteworthy and reliable that has been published to date. The amboceptor and complement are both titrated daily and two units of each used in the tests. The complement is titrated in the presence of antigen. I have employed this technic, modified in one detail which is considered important, for a number of years and have found it a most satisfactory test for the detection of syphilitic reagin.

The modification above referred to is the addition of a small amount of Wassermann-negative human serum to each tube employed in the complement titration along with the other reagents employed. Table I serves to show the reagents employed in the complement titration.

TABLE I

TUBE	1	2	3	4	5	6	7	8	9	10	11
	c.c.	c.c	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	
Complement	0.5	0.45	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.05	0
Physio. Salt Sol.	0.9	0.9	1.0	1.0	1.1	1.1	1.2	1.2	1.3	1.3	2.5 c.c.
Antigen 10 units	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
Normal Human Serum	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0
Place in water-bath at 37° C. for one-half hour and add:											
Hemolytic Amboceptor 2 units	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0
2% Suspension Sheep's R.B.C.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

This technic was suggested by Kolmer in his paper: "Studies in the Standardization of the Wassermann Reaction XI,"[8] but was not accepted by him because the unit obtained was too small if fresh sera, free of anticomplementary activity, were used, and gave serum controls that were not completely hemolyzed. This difficulty, however, has not been experienced. In fact with two units of amboceptor and two units of complement as indicated by this titration, which is less than that obtained by the plain titration (with no serum added), the hemolytic and serum controls are always perfectly clear in thirty to forty minutes. Kolmer states in his paper, "Studies in the Standardization of the Wassermann Reaction XX,"[9] that the very rapid or "explosive" type of hemolysis is not as desirable as an adjustment of the hemolytic system which will produce complete hemolysis in one-half to one hour, because it is apt to reduce the sensitiveness of reactions with weakly positive sera.

Table II shows the full unit, as described by Kolmer, to be the amount in tube No. 5 in the plain titration and No. 6 in the titration with normal serum. In the first instance the dose (2 units) of complement indicated is 1 c.c. of a 1:50 dilution; in the second, 1 c.c. of a 1:60 dilution. The difference in amount of actual guinea pig serum represented in these two tubes is

the difference between 1/50 (6/300) and 1/60 (5/300) of a cubic centimeter, or 20 per cent less in the dose when titrated in the presence of normal serum. This difference is realized in the tests by the difference in rapidity of hemolysis. The larger dose produces a noticeable discoloration due to hemolysis within three or four minutes and hemolysis is complete and the tubes sparkling clear in eight to ten minutes; whereas the smaller dose produces signs of hemolysis in twelve to fifteen minutes and clear tubes in one-half hour. Kolmer[10] attributes this increase in hemolytic activity to the presence of natural antisheep hemolysis in the normal human serum whenever 0.1 c.c. or more of the serum is employed, but believes that when less than 0.1 c.c. amounts this is a negligible factor.

TABLE II

COMPLEMENT TITRATIONS

PLAIN TITRATION AND ONE WITH SERUM ADDED

TUBE	1	2	3	4	5	6	7	8	9	10	DIL. OF COMP. FOR DOSE OF 1 C.C. CONTAINING 2 UNITS
Amt. of Comp. 1:30 dil.	c.c. 0.5	c.c. 0.45	c.c. 0.4	c.c. 0.35	c.c. 0.3	c.c. 0.25	c.c. 0.2	c.c. 0.15	c.c. 0.1	c.c. 0.45	
Plain* No serum	0	0	0	0	0 full unit	0	+	+ + +	+ + + +	+ + + +	1:50
Normal x Serum	0	0	0	0	0	0 full unit	0	+	+ +	+ + + +	1:60

*Plain titration is one with complement, saline and antigen, but no serum.
0 = no fixation of complement; + + + + = complete fixation.
x Normal serum is a pooled specimen from 4 or 5 individuals.

However, after observing this action with almost absolute regularity for several months and obtaining what was thought to be a superior complement-fixation test by the use of a smaller complement dose, it was decided to try to ascertain, if possible, just what ingredient or ingredients were responsible for the increased hemolytic power.

EXPERIMENTAL

The first experiment was to collect blood specimens from a number of the domesticated animals and determine whether their blood possessed the same peculiarity toward the hemolytic system as did the human serum. Table III shows the results of this experiment.

It is readily seen from Table III that the serum of the ox and guinea pig as well as the normal human serum possess some property which gives greater hemolytic power to the complement than is possessed without them.

Now, to answer the question of whether this property is not specific antisheep amboceptor, native in the blood stream of the species in question. Guinea pig blood being too precious to use for experimentation, only the ox and human sera were tested out further.

Sera from these two were subjected to absorption of antisheep hemolysin by sheep's red blood corpuscles. Two drops of packed sheep's red blood cells, from the end of an ordinary 1 c.c. serologic pipette, were put into a test tube containing about 2 c.c. of the serum to be absorbed, the tube gently

TABLE III

ONE-TENTH C.C. OF EACH SERUM (HEATED FOR THIRTY MIN. AT 55° C.) ADDED WHEREVER INDICATED

TUBE	1	2	3	4	5	6	7	8	9	10	DIL. OF COMP. FOR DOSE OF 1 C.C. CONTAINING 2 UNITS
Complement	c.c. 0.5	c.c. 0.45	c.c. 0.4	c.c. 0.35	c.c. 0.3	c.c. 0.25	c.c. 0.2	c.c. 0.15	c.c. 0.1	c.c. 0.05	
Plain*											
No serum	0	0	0	0	0	+	++	++++	++++	++++	1:42.9
Sheep serum	0	0	0	++	+++	++++	++++	++++	++++	++++	1:33.3
Human serum	0	0	0	0	0	0	+	+++	++++	++++	1:50
Goat serum	0	0	0	0	+	++++	++++	++++	++++	++++	1:37.5
Rabbit serum	0	0	+	++	+++	++++	++++	++++	++++	++++	1:30
Ox serum	0	0	0	0	0	0	0	++	+++	++++	1:60
Guinea pig serum	0	0	0	0	0	0	+	++	++++	++++	1:50

*Plain titration = one with antigen but no serum added.
0 = no fixation of complement; ++++ = complete fixation.

shaken to make a uniform suspension, and allowed to stand at room temperature for twenty to thirty minutes before centrifuging. Tubes were shaken once or twice during this interval to keep cells in suspension. Cells were removed by centrifugalization and the sera heated for one-half hour at 55° C. Potent immune amboceptor absorbed in this manner for ten minutes was practically inactive, showing very slight hemolysis in a dose five times as large as the unit of the active serum. Table IV will show the contrast. In Table IV is shown the effect of the addition of serum, with and without

TABLE IV

TITRATION OF ANTISHEEP AMBOCEPTOR, BEFORE AND AFTER ABSORBING (TEN MINUTES) WITH SHEEP'S CORPUSCLES

Dilution Is 1:3000

TUBE	1	2	3	4	5	6	7	8	9	10
Active amboceptor	c.c. 0.5	c.c. 0.45	c.c. 0.4	c.c. 0.35	c.c. 0.3	c.c. 0.25	c.c. 0.2	c.c. 0.15	c.c. 0.10	c.c. 0.05
Result*	0	0	0	0	0	0	0	+	++	+++
Absorbed amboceptor	1.0	0.5	0.4	0.3	0.2	0.1	–	–	–	–
Result	+++	++++	++++	++++	++++	++++				
Complement	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
2 per cent sheep cells	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

*0 = no fixation of complement; ++++ = complete fixation.

absorption of antisheep hemolysin to each tube used in the titration of complement. Such experiments, with the human serum, were continued over a period of about one year and a large number were performed, the foremost thought at each performance being to further substantiate the evidence which had been gained or else to pick up a thread of disproving evidence. Occasionally a specimen of the human serum caused no greater degree of hemolysis than without serum, and in only a very few instances (perhaps 3 or 4) did it actually retard hemolysis. The human serum was a pooled specimen of from 3 to 6 or 7 individual specimens that were left over from the previous day's run of Wassermanns. Care was taken to select only those specimens that were free of bacterial growth and excessive hemolysis, and gave

a clear sparkling appearance before an electric light. All tests for the Wassermann reaction were run within forty-eight hours of the time the blood was drawn; hence the advisability of selecting uncontaminated sera for use in the adjustment of the hemolytic system. Otherwise the antilytic effect of bacteria would more than offset the favorable action of the normal serum and obscure

TABLE V

COMPLEMENT TITRATION COMPARING PLAIN TITRATION, AND THOSE WITH SERUM, AND ABSORBED SERUM

TUBE	1	2	3	4	5	6	7	8	9	10	DIL. OF COMP. FOR DOSE OF 1 C.C. CONTAINING 2 UNITS
Complement	c.c. 0.5	c.c. 0.45	c.c. 0.4	c.c. 0.35	c.c. 0.3	c.c. 0.25	c.c. 0.2	c.c. 0.15	c.c. 0.1	c.c. 0.05	
Plain* titration	0	0	0	0	0	+	++	+++	++++	++++	1:42.9
Human serum 0.1 c.c.	0	0	0	0	0	0	+	+++	+++	++++	1:50
Absorbed H. serum 0.1 c.c.	0	0	0	0	0	0	+	++	++++	++++	1:50
Ox serum 0.1 c.c.	0	0	0	0	0	0	+	+	++	++++	1:50
Absorbed ox serum 0.1 c.c.	0	0	0	0	0	0	0	+	++	++++	1:60

*Plain titration = one with antigen but no serum added.
0 = no fixation of complement; + + + + = complete fixation.

TABLE VI

RESULTS OF DIFFERENT AMOUNTS OF NORMAL HUMAN SERUM ON THE COMPLEMENT TITRATION

TUBE	1	2	3	4	5	6	7	8	9	10	DIL. OF COMP. FOR DOSE OF 1.0 C.C. CONTAINING 2 UNITS
Complement	c.c. 0.5	c.c. 0.45	c.c. 0.4	c.c. 0.35	c.c. 0.3	c.c. 0.25	c.c. 0.2	c.c. 0.15	c.c. 0.1	c.c. 0.05	
Plain* No serum	0	0	0	0	0	+	+++	+++	++++	++++	1:42.9
Antigen + 0.002 c.c. normal serum	0	0	0	0	0	+	++	+++	++++	++++	1:42.9
Antigen + 0.005 c.c. normal serum	0	0	0	0	0	+	+	+++	++++	++++	1:42.9
Antigen + 0.01 c.c.	0	0	0	0	0	0	+	++	++++	++++	1:50
Antigen + 0.05 c.c.	0	0	0	0	0	0	+	+	+++	++++	1:50
Antigen + 0.1 c.c.	0	0	0	0	0	0	+	++	++++	++++	1:50
Antigen + 0.2 c.c.	0	0	0	0	0	0	+	++	+++	++++	1:50
Antigen + 0.3 c.c.	0	0	0	0	0	0	++	++	++++	++++	1:50
Antigen + 0.4 c.c.	0	0	0	0	0	+	++	+++	++++	++++	1:42.9
Antigen + 0.5 c.c.	0	0	0	0	+	+	++	++++	++++	++++	1:37.5

*Plain titration = one with antigen but no serum added.
0 = no fixation of complement; + + + + = complete fixation.

the results. Ox serum was secured from the abbatoir and was probably all obtained from one beef. However, only a few tests were made with the ox blood, the same type of results being obtained whenever it was used.

Not only does the 0.1 c.c. portion of normal serum (that amount which is employed as the maximum dose and for the serum control in the Kolmer-Wassermann test) lend the augmentary effect in the hemolytic system, but any amount from 0.01 c.c. up to 0.3 c.c. produces the same result.

It is evident from Table VI that only a minute amount of the unknown reagent is required to cause increased hemolytic activity, and also that quite large amounts of normal serum produce no effect on the hemolytic system.

So far, the saline solution employed was 0.85 per cent sodium chloride in distilled water, no buffer salts having been tried. The use of a buffered saline was considered and the formula recommended by Mason and Sanford[11] given a trial. This is made of sodium chloride 7.0 gm., secondary sodium phosphate 1.7 gm., and primary potassium phosphate 0.2 gm. to each liter of distilled water. This caused slight hemolysis of freshly drawn and washed sheep's corpuscles within one hour in the 38° C. water-bath, and marked hemolysis was noted after standing overnight in the refrigerator.

A further search into the literature revealed that Manwaring[12] and Hektoen and Ruediger[13] in 1904 had conducted extensive experiments relative to the behavior of red blood corpuscles and immune amboceptors and the complements of animal serum when subjected to the action of weak solutions of a great variety of inorganic salts. They came to the conclusion that many of the inorganic salts in small doses prevent hemolysis due to their action on the complements; a certain number produce marked hemolysis of red blood corpuscles, while others are less hemolytic.

Kellogg and Wells[14] found that the magnesium salts, $MgCl_2$ and $MgSO_4$, in minute quantities (0.01 per cent) possessed the power of enhancing the hemolytic titer of immune serum to almost three times that shown with the use of plain physiologic saline in distilled water. This solution was given a trial for a short period but its use soon discontinued because the results seemed too variable, contrary to the report of Kellogg.

The next step was to ascertain, if possible, just what fraction or fractions of the normal human serum was responsible for the production of the phenomenon in question. The first thought was to separate the globulins in as near a pure state as possible and determine if they were identifiable as the causative agent.

Methods of obtaining globulin solutions from horse serum for experimental purposes were described by Mellanby[15] and Hardy[16] in 1905. They believed that globulin precipitated by neutral salts and redissolved in weak salt solutions retained all the properties of the original substance; but if exposed to prolonged contact with distilled water, the properties were materially altered.

Howe[17] in 1921 published a method for removal of the different globulin fractions from blood serum by precipitating with specified concentrations of anhydrous sodium sulphate solution. This was the method employed in these

experiments. After precipitation the salt was dialyzed through collodion membranes as prepared by Brown[18] in his experiments on grading collodion membranes with alcohols, except that DuPont's collodion was used instead of Schering's and larger sacs were made, using 250 c.c. Erlymeyer flasks instead of test tubes for forms.

Preparation of Collodion Sacs.—Dissolve 8 gm. of DuPont's "Parlodion" in a solution of absolute alcohol and ether, equal parts by volume. Pour about 10 c.c. (a little more or less may be required which will be learned from experience in making a few sacs) of this into a clean Erlymeyer flask the size of the sac desired. Insert a rubber stopper to prevent too rapid evaporation and rotate the flask at such an angle as will allow the collodion to flow gently toward the mouth of the flask. When the entire surface of the flask is covered, remove stopper and allow to dry for four or five minutes. Then fill the flask with water from the tap, running it in slowly at first so as not to mar the sac which is yet quite soft. Pour the water out after a few minutes and fill the flask about half full of 90 per cent alcohol. With a knife begin at the mouth of the flask to peel the membrane from the glass. As soon as a little space is made between membrane and glass fill it with 90 per cent alcohol and continue to peel and add alcohol to moisten the outside of the sac until it collapses. Then pour the alcohol out and carefully draw the sac out through the mouth of the flask. Wash the sac in water and allow to dry overnight at room temperature. Now soak the air-dried membrane for twenty-four hours in 80 per cent (by volume) alcohol at 20° C., or ordinary room temperature. Transfer to distilled water and wash for twenty-four hours, after which it may be kept in water until ready to be used. This membrane is said to have an "alcoholic index" of 80 and will allow rapid passage of crystalloids but retains all colloids.

Technic of Preparing Globulin Solutions.—The three kinds of globulin obtained and described by Howe are: (1) euglobulin, precipitated by 13.5 per cent sodium sulphate solution; (2) pseudoglobulin I, precipitated along with euglobulin by 17.4 per cent of the salt; and (3) pseudoglobulin II is included in the precipitate of a 21.5 per cent solution. Precipitation was carried out in the incubator at 37° C. by adding 10 c.c. of the salt solution to each cubic centimeter of serum. Thus, 10 c.c. of serum are put into a 200 c.c. glass-stoppered bottle and 100 c.c. of the sodium sulphate solution added, mixed and allowed to stand for about fifteen hours (overnight). The following morning the supernatant liquid is siphoned off leaving the flaky, loose precipitate in the bottom with as little of the salt solution as possible. Now the remaining contents are shaken gently and transferred to the collodion sac, which is stoppered with a cork, held in place by tying with a string around the top of the membrane. The sac may be filled nearly full with distilled water which will make it easier to keep submerged and also hasten the dialysis. It is now placed in a large container (14 quart enamel water pail was used and the water changed after two or three hours) of distilled water and allowed to dialyze for twelve to fifteen hours. Remove the globulin, which is now suspended in distilled water, to several large, thick walled, Pyrex tubes for centrifugalization. Centrifuge at high speed, 3000 r.p.m. or

more, for twenty to thirty minutes. Decant the supernatant fluid and take up the entire amount of globulin in 5 c.c. of physiologic saline. Not all the globulin will be dissolved, but sufficient will go in solution to give a protein test with Exton's sulphosalycilic acid precipitation method of 400 to 600 mg. per 100 c.c. of solution, and that is sufficient, with the precipitate of the higher salt concentrations, to produce the same reaction on the complement titration as does the whole serum itself. This result may be shown in a more comprehensive manner by the use of a comparative table. (Table VII.)

TABLE VII

COMPARISON OF RESULTS OF ADDING SEPARATED SERUM GLOBULINS, WHOLE SERUM AND NO SERUM TO COMPLEMENT TITRATION

TUBE	1	2	3	4	5	6	7	8	9	10	DIL. OF COMP. FOR DOSE OF 1 C.C. CONTAINING 2 UNITS
Plain* No serum	0	0	0	0	0	+	++	+++	+++	++++	1:42.9
Normal serum	0	0	0	0	0	0	+	++	+++	++++	1:50
Absorbed serum	0	0	0	0	0	0	+	++	+++	++++	1:50
17.4 per cent globulin	0	0	0	0	0	0	+	+	++	++++	1:50
21.5 per cent globulin	0	0	0	0	0	0	+	+++	++++	++++	1:50

*Plain titration = one with antigen but no serum added.
17.4 per cent glob. = globulin precipitated by 17.4 per cent Na_2SO_4 solution.
21.5 per cent glob. = globulin precipitated by 21.5 per cent Na_2SO_4 solution.
0 = no fixation of complement; + + + + = complete fixation.

The euglobulin, or the fraction precipitated by the use of 13.5 per cent sodium sulphate, increased the activity but slightly over the plain titration so was not included in the table for that reason.

SUMMARY

1. A method is presented for the more delicate adjustment of the hemolytic system employed in the Kolmer-Wassermann reaction. This is accomplished by the addition of a small amount of uncontaminated normal human serum, along with the complement, saline and antigen, to the tubes employed in the titration of complement.

2. This more nearly simulates the conditions in the tubes of the actual tests, because allowance is made for any affinity the serum proteins may have for complement.

3. Amounts of serum too small to be regarded as containing appreciable amounts of antisheep hemolysin are capable of producing increased hemolysis in the complement titration.

4. Human serum deprived of its antisheep hemolysin by absorption with sheep's corpuscles is capable of producing increased hemolysis in the complement titration to the same degree as a portion of the same specimen of serum untreated.

5. Serum from the guinea pig and ox enhanced the action of complement in the titration, the ox serum being more potent than human serum. Sera of the goat, sheep, dog and rabbit possessed inhibitory properties.

6. Serum globulins obtained by salting out and dialysis enhanced the reaction to the same degree as did the serum itself.

7. The technic is given for the preparation of collodion sacs and for separating the globulins from blood serum.

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LABORATORY METHODS

A CLINICAL METHOD FOR THE QUANTITATIVE DETERMINATION OF PANCREATIC FERMENTS IN DUODENAL CONTENTS*

By Edward Hollander, B.S., M.D., New York City

IN A PREVIOUS paper,[1] a simple method for the quantitative determination of pancreatic ferments was described. The activity of steapsin and trypsin was found to be inversely proportional to the time required for the neutralization of 1 c.c. of tenth normal sodium hydroxide by the fatty and amino acids formed by the hydrolysis of olive oil or gelatine. The activity of amylopsin was proportional to the amount of maltose formed by the hydrolysis of starch. In the present paper a technic for the collection of duodenal fluid and its quantitative examination for pancreatic ferments will be described which has been found to result in consistent values for the ferments in normal cases.

Collection of Sample of Duodenal Contents.—The pancreatic ferments are discharged into the duodenum through the ducts of Wirsung and of Santorini. The duct of Wirsung, which is the main pancreatic duct, joins the common bile duct and the pancreatic fluid is discharged with bile through the ampulla of Vater. The duct of Santorini is a small and variably developed duct which is not infrequently absent, but when present enters the duodenum about two centimeters from the ampulla.

To insure relaxation of the duodenum to permit pancreatic juice to flow from the ducts, three injections of one ounce of 33 per cent aqueous solution of magnesium sulphate are given through a duodenal tube at ten-minute intervals. The bile colored flow from the duodenal tube is collected in test tubes, about 5 c.c. in each tube. The specimen having the deepest color is selected for examination as it was found that the deepest colored tube contained the pancreatic ferments in highest concentration. The tube selected is placed in cracked ice and is examined within eight hours as steapsin is very unstable at room temperature and when iced shows diminution in activity after eight hours.

TECHNIC

Steapsin.—To 50 c.c. of olive oil† emulsion ½ c.c. of 1 per cent alcoholic solution of phenolphthalein is added. Tenth normal sodium hydroxide is slowly added to the emulsion until a definite faint pink color is formed.

*From the Manhattan State Hospital.
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†Olive Oil Emulsion (10 per cent): Place 50 gm. of gum arabic in a mortar and add 50 c.c. of pure olive oil and rub into a smooth paste. Then add 50 c.c. of distilled water, constantly stirring, until a white emulsion is formed. Gradually add more water to a total volume of 500 c.c. Keep in ice box.

Twenty-five c.c. of this neutralized oil is placed in each of two 30 c.c. test tubes. To one of these tubes 1 c.c. of tenth normal sodium hydroxide is added which produces a deep red and the second tube is used as a control. The tubes are placed in a jar of water at 37.5° C. and this temperature is maintained by the addition of hot water as required. To both tubes 0.1 c.c. of duodenal contents is added from a 1 c.c. pipette. The test tube is then shaken to insure even distribution of the enzyme within the substrate. The time of addition of the ferment is noted and again the time when all pink disappears by comparison with the control tube. In normal cases this occurs within five minutes. For clinical purposes, in delayed reactions the test need not be observed longer than twenty minutes. The ferment activity being inversely proportional to the time of the reaction its strength may be expressed as a fraction of the normal by dividing five minutes by the time of the reaction:

$$\text{Strength of Steapsin} \quad \frac{\text{5 minutes}}{\text{Time of reaction in minutes}}$$

Trypsin.—The technic of examination is identical with that for steapsin except that 0.5 c.c. of duodenal contents is employed and 5 per cent gelatine* in an emulsion of mineral oil is used as substrate. The disappearance of all pink by comparison with the control tube occurs within five minutes in normal cases.

$$\text{Strength of Trypsin} \quad \frac{\text{5 minutes}}{\text{Time of reaction in minutes}}$$

Amylopsin.—To a beaker containing 50 c.c. of 3 per cent aqueous solution of soluble starch, ½ c.c. of duodenal contents is added. This is incubated for one-half hour at 37.5° C. To stop further activity of the amylopsin a small amount of sodium carbonate is added. The number of cubic centimeters of digestion mixture required to reduce 5 c.c. of Benedict's quantitative sugar reagent is determined, which in normal cases is 1.9 c.c. or less. The activity of amylopsin being proportional to the amount of maltose formed and inversely proportional to the amount of the digestion mixture required to reduce the Benedict's solution, in cases of diminished amylopsin activity the strength of the ferment may be expressed as a fraction of the normal:

$$\text{Strength of Amylopsin} \quad \frac{\text{1.9 c.c.}}{\text{c.c. of substrate required to reduce 5 c.c. of Benedict's solution}}$$

*Gelatine Emulsion (15 per cent): This emulsion is milky white and forms an excellent contrast medium for the pink color of the phenolphthalein. Aqueous gelatine solution is of a light brown color and does not contrast well with a pink color.

The emulsion is made as follows: Add 25 gm. of highest grade gelatine to 300 c.c. of distilled water and gradually heat to 60° C. until the gelatine is completely dissolved.

To 50 gm. of best grade (whitest grade) gum arabic in a mortar add 10 c.c. of distilled water and rub into a smooth paste. Then add 50 c.c. of liquid albolene and keep rubbing into a smooth paste. Then gradually add 40 c.c. of distilled water with continual stirring. When a homogenous paste is formed add the heated gelatine solution gradually and stir continually. A milky white emulsion is then formed. Add hot distilled water slowly until the total volume is 500 c.c. Keep in ice box in 50 c.c. flasks. When needed these are liquefied by gentle heat in a water-bath.

Table I gives the normal findings for steapsin, trypsin and amylopsin in 40 tests on 35 normal cases.

Table II is a list of normal findings for steapsin, trypsin and amylopsin in 18 tests on 11 cases of various gastrointestinal diseases.

TABLE I

NORMAL FINDINGS FOR STEAPSIN, TRYPSIN, AND AMYLOPSIN IN 35 NORMAL CASES

PATIENT	STEAPSIN, MINUTES	TRYPSIN, MINUTES	AMYLOPSIN, C.C.
D. A.	2	4	1.3
A. B.	2¼	3	1.4
M. C.	3	2	1.9
H. E.	1¾	3	1.4
J. E.	5	5	1.3
S. F.	3	5	1.5
R. G.	4½	5	1.5
B. G.	2	1	1.4
E. G.	4	3	1.3
Repeated 2 weeks later	5	4½	1.9
A. G.	3	2	1.4
E. S.	2½	3½	1.9
M. J.	4½	4½	1.4
A. K.	1½	2½	1.6
F. K.	1	1	1.5
Repeated 1 month later	2½	3	1.6
S. L.	3¾	5	1.9
B. L.	5	5	1.6
E. M.	1	1½	1.3
H. N.	5	5	1.7
J. C.	2	2½	1.3
M. O.	1	2	1.2
I. P.	5	5	1.7
E. R.	3½	4½	1.2
C. W.	4	2½	1.9
W. W.	2½	3	1.5
A. T.	5	4	1.4
N. S.	3½	2	1.9
C. S.	4	4½	1.7
S. S.	3½	5	1.9
B. S.	1¼	1¾	1.2
Repeated 3 weeks later	1¼	1¼	1.2
5 weeks later	5	3	1.4
7 weeks later	2¾	5	1.4
9 weeks later	1½	2	1.4
H. C.	1½	3	1.9
M. C.	3½	4	1.6
B. D.	1¾	2¼	1.2
M. G.	2	2½	1.3
I. G.	1¼	1½	1.7
Repeated 1 day later	2	2	1.7

TABLE II

NORMAL FINDINGS FOR THE PANCREATIC ENZYMES IN 11 CASES OF INTRAABDOMINAL DISEASE

PATIENT	STEAPSIN MINUTES	TRYPSIN MINUTES	AMYLOPSIN C.C.	DIAGNOSIS
C. A.	3	4	1.7	Duodenal ulcer
S. A.	2	5	1.9	Gallstones
A. B.	3½	4½	1.7	Cholesterosis of gall bladder
W. H.	1	2¾	1.4	Duodenal ulcer
1 month later	1½	2½	1.7	
2 months later	1½	2½	1.4	
F. G.	1½	3	1.7	Duodenal ulcer
1 month later	4	1¾	1.5	
3 months later	2	2½	1.5	
G. K.	2	3½	1.1	Gastric syphilis
R. M.	2½	3½	1.9	Mucous colitis
M. M.	2	4	1.7	Duodenal ulcer
2 months later	2½	4	1.3	
N. P.	2	3¼	1.5	Duodenal ulcer
2 weeks later	4¾	5	1.8	
W.	5	5	1.7	Gastric ulcer
A. S.	2	2	1.2	Duodenal ulcer
3 months later	1	1½	1.1	

Table III is a list of 21 cases in which delayed activity of one or more of the ferments was obtained.

COMMENT (TABLE III)

Only steapsin was diminished in Cases 10 and 11. In Case 11 cholelithiasis was present. In Case 10 the symptoms consisted of midepigastric pressure and belching soon after meals. No other abnormal finding was obtained in studies of the gastrointestinal tract. Elimination of fat resulted in a speedy relief from symptoms.

In Case 13 only amylopsin was diminished. The symptoms consisted of abdominal distension and frequent unformed bowel movements. Studies of the gall bladder, stomach, duodenum and colon were negative for organic disease. The symptoms disappeared by the elimination of carbohydrates from the diet.

Of the eleven cases showing all three ferments reduced, grave conditions were present in seven cases that showed marked reduction in the strength of the ferments (Cases 4, 5, 9, 17, 20, 21, and 22). Cases 1 and 16 occurred with gallstones. In Case 2 gastric anacidity had existed for many years and cholecystectomy had been done for cholesterosis of the gall bladder. In Case 8 studies of the gall bladder, stomach, duodenum, and colon were negative for organic disease. The symptoms consisted of pyrosis, abdominal distention, alternating constipation and loose bowel movements. The diminution in pancreatic ferment activity was the only finding obtained to account for the symptoms present.

In the remaining eight cases showing diminution in only two ferments, gallstones were present in three cases (Cases 3, 12, and 14) and in Case 14 carcinoma of the liver was also found. In Case 19 mild icterus due to hep-

TABLE III

CASES WITH DIMINISHED FERMENTS. (STRENGTH OF FERMENT EXPRESSED AS PERCENTAGE OF NORMAL.)

PATIENT	STEAPSIN MINUTES	TRYPSIN MINUTES	AMYLOPSIN C.C.	DIAGNOSIS
(1) M. R.	20 = 25%	10 = 50%	2.0 = 95%	Gallstones 10 yr. duration)
6 days later	17 = 29%	9 = 55%	2.0 = 95%	
(2) T. G.	8 = 62%	10 = 50%	2.2 = 86%	Gastric anacidity
(3) E. H.	3	8 = 62%	2.0 = 95%	Gallstones
(4) J. J.	20 = 25%	20 = 25%	4.2 = 45%	Cirrhosis of liver
(5) B. M.	At 20 min. reaction not complete = less than 25 per cent	15 = 33%	8.5 = 22%	Gallstones (14 yr. duration)
(6) A. L.	8 = 62%	6 = 83%	1.7	Duodenal ulcer
(7) H. W.	7 = 71%	7 = 71%	1.5	Duodenal ulcer
(8) L. B.	No reaction in 20 min.	12 = 41%	2.3 = 83%	
7 days later	No reaction in 20 min.	10 = 50%	2.0 = 95%	
(9) F. S.	12 = 42%	13 = 39%	2.3 = 83%	Cirrhotic liver
(10) H. S.	11 = 45%	4.5	1.4	
3 days later	8 = 62%	4	1.8	
(11) M. N.	12 = 42%	3	1.2	Gallstones (8 yr. duration)
2 days later	10 = 50%	2.5	1.3	
(12) J. M.	7 = 71%	3	2.6 = 73%	Gallstones
2 days later	10 = 50%	5	3.2 = 59%	
(13) M. S.	3.5	1	3.4 = 55%	
3 days later	5	2	4.2 = 45%	
(14) F. M.	5	12 = 41%	2.3 = 85%	Carcinoma of liver with gallstones
12 days later	4	13 = 39%	2.6 = 73%	
(15) H. R.	10 = 50%	5	4.2 = 45%	
3 days later	10 = 50%	5	3.2 = 59%	
(16) L. S.	15 = 33%	15 = 33%	Absent	Gallstones
(17) R. G.	At 20 min. reaction not complete = less than 25 per cent	20 = 25%	2.9 = 65%	Gallstones with cirrhotic liver
(18) J. B.	9 = 56%	4	2.0 = 95%	Nephrosis
(19) G. R.	15 = 33%	7 = 71%	1.7	Hepatitis
(20) C. E.	Absent: no reaction in 20 min.	Absent: no reaction in 20 min.	Absent: no reduction of Benedict's reagent with 10 c.c. of substrate	Cirrhosis of liver with jaundice
(21) M. B.	20 = 25%	20 = 25%	5 = 38%	Carcinoma of liver with jaundice
(22) T. G.	Absent: no reaction in 20 min.	At 20 min. reaction not complete = less than 25 per cent	5 = 38%	Pancreatitis with cholelithiasis

atitis following cholecystectomy for gallstones was present. Duodenal ulcer was present in two cases (Cases 6 and 7). Case 18 had nephrosis with diarrhea which resisted the usual method of treatment with bismuth and opium. On a fat-free diet and the use of pancreatin keratin coated capsules, the diarrhea was quickly controlled. The patient in Case 15 complained of pyrosis and flatulence. The diminution in steapsin and amylopsin was the only abnormal finding obtained in the study of the gastrointestinal tract. Regulation of the diet by the elimination of fats and the reduction of carbohydrates resulted in the disappearance of symptoms.

CONCLUSIONS

1. A standard technic for the collection of a specimen of duodenal contents and a simple method for the quantitative determination of pancreatic ferments is described.

2. The tests for the activity of steapsin and trypsin depend upon the time required for the fatty acids or the amino acids formed to neutralize 1 c.c. tenth normal sodium hydroxide which in normal cases occurs within five minutes.

3. The test for the activity of amylopsin depends upon the amount of substrate (containing maltose) required to reduce 5 c.c. of Benedict's quantitative sugar reagent. In normal cases the reduction occurs with 1.9 c.c. or less.

4. In cases of diminished ferment activity, simple equations are given to express the strength of the ferments quantitatively as fractions or percentages of the normal.

5. In the cases with diminished pancreatic ferments either one or combinations of the three enzymes was affected. In most of the cases the diminished pancreatic activity was associated with disease of the gall bladder or liver.

In five patients the diminished pancreatic activity was the only finding obtained in the study of the gastrointestinal tract to account for the symptoms present.

I wish to express my appreciation for the cooperation of Roberta Bennett, R. N., in carrying out this work.

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THE USE OF THE RODELLA PUTRIMETER IN A STUDY OF INTESTINAL PUTREFACTION*

By H. B. Pierce,† Ph.G., Rochester, N. Y.

IN 1912 Rodella[1] devised a simple test intended for the quantitative determination of intestinal putrefaction. A flask containing egg albumen is inoculated with a small amount of feces from a carefully mixed sample. The contents of the flask are stirred, and the flask is heated at a temperature of 85° to 90° for fifteen minutes. The flask is then fitted with a sterile one-hole rubber stopper, having a glass tube outlet plugged with cotton, and connected to a mercury manometer, the flask and manometer now being placed in an incubator. The level of the mercury is adjusted after a constant temperature is reached. After twenty-four hours, the gas pressure is noted and the flask is removed from the incubator. The liquefied egg albumen is measured and the reaction and odor of the contents noted. Rodella formulated a putrefactive index expressed as follows:

$$\frac{\text{Mm. Hg} \times \text{c.c. fluid egg white}}{\text{Number of hours of incubation}} = \text{"Faulniss" index.}$$

Von Deschwanden[2] has used Rodella's method, and has found high putrefactive indices accompanying constipation.

During the course of a study on the effect of yeast ingestion on intestinal putrefaction, the results of which are to be published elsewhere, use was made of Rodella's method in an effort to determine whether there was a relationship between results obtained by this method and the usual chemical indices of intestinal putrefaction. Two diets, which were carefully controlled, were used with four subjects during the course of this investigation. One diet was relatively high in protein and the other relatively high in carbohydrate, but neither diet was abnormal in any sense of the word. Each diet was divided into four periods, two serving as control periods and two as yeast periods as set forth in the tables.

The following foods comprised the menu in diet II ("high protein"):

Breakfast:	Orange juice or banana, corn flakes or shredded wheat with cream and sugar, one egg boiled or fried, toast, butter, jam, and coffee with cream and sugar.
Luncheon:	Boiled ham, two poached eggs on toast, butter, gelatin dessert, and cream.
Dinner:	Beef steak, mashed potatoes, string beans, bread and butter, ice cream, and three subjects had coffee with cream and sugar.

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†With the assistance of D. D. Posson and M. S. Pierce.

TABLE I

DIET II. "HIGH PROTEIN"*

PERIOD	DAY	SUBJECT D. GAS MM. HG	SUBJECT D. LIQ. C.C.	SUBJECT D. NO. OF STOOLS	SUBJECT H. GAS MM. HG	SUBJECT H. LIQ. C.C.	SUBJECT H. NO. OF STOOLS	SUBJECT M. GAS MM. HG	SUBJECT M. LIQ. C.C.	SUBJECT M. NO. OF STOOLS	SUBJECT P. GAS MM. HG	SUBJECT P. LIQ. C.C.	SUBJECT P. NO. OF STOOLS
	1	0	0	0	17	2	1	0	2	1	27	4	1
	2	3	4	1	22	3	1	117	8	1	61	5	1
First	3	0	0	0	2	1	1	171	3	1	53	2	1
Control	4	5	0	1	3	0	1	150	6	1	48	4	1
	5	121	8	1	40	1	2	180	5	1	145	10	1
	6	54	2	1	42	2	2	225	5	1	165	5	1
	Av.	46	3.5		21	1.5		141	5		83	5	
	1	20	2	1	37	2	2	82	4	1	155	3	1
Three	2	0	0	0	69	0	2	69	0	1	69	0	2
Yeast	3	10	0	1	22	0	1	4	0	1	82	0	1
Cakes	4	26	0	1	52	0	1	50	0	1	60	0	1
Per	5	38	0	1	50	0	1	42	0	1	8	0	2
Day	6	40	1	1	52	1	2	75	2	1	178	5	2
	Av.	27	0.6		47	0.5		54	1		92	1.3	
	1	45	0	1	27	0	1	116	2	1	52	2	1
	2	–	–	–	–	–	–	–	–	–	–	–	–
Second	3	30	0	1	20	0	1	65	0	1	132	0	1
Control	4	22	1	1	24	2	1	90	1	1	109	3	1
	5	20	0	1	4	0	1	65	2	1	72	2	1
	6	35	0	1	5	0	2	102	0	1	65	0	1
	Av.	30	0		16	0.4		88	1		86	1.4	
	1	51	0	1	25	0	2	46	0	1	50	0	1
Six	2	18	0	1	70	0	1	108	2	1	147	2	1
Yeast	3	46	0	1	81	0	2	140	0	1	67	0	2
Cakes	4	78	0	1	70	0	1	80	0	1	123	0	1
Per	5	38	0	1	67	0	1	130	0	1	85	0	1
Day	6	23	0	1	28	0	1	20	0	1	60	0	1
	Av.	42	0		57	0		87	0.3		89	0.3	

*No corrections were made for changes in barometric pressure.

TABLE II

AVERAGE CHEMICAL INDICES OF PUTREFACTION FOR SIX-DAY PERIODS
DIET II—"HIGH PROTEIN"

PERIOD	SUBJECT D. TOTAL PHENOLS MG.	SUBJECT D. INDOLE MG.	SUBJECT D. INDICAN	SUBJECT H. TOTAL PHENOLS MG.	SUBJECT H. INDOLE MG.	SUBJECT H. INDICAN	SUBJECT M. TOTAL PHENOLS MG.	SUBJECT M. INDOLE MG.	SUBJECT M. INDICAN	SUBJECT P. TOTAL PHENOLS MG.	SUBJECT P. INDOLE MG.	SUBJECT P. INDICAN
First Control Three Yeast Cakes Per Day	616	4.4	+++	704	7.0	++	346	3.4	+	483	1.5	++
Second Control Six Yeast Cakes Per Day	633	8.5	++++	741	9.0	+++	376	3.8	++	560	4.4	++
	570	5.4	+++	675	6.2	+++	359	2.5	++	474	2.6	+++
	597	7.0	++++	750	15.6	+++	412	3.1	++	582	5.2	+++

The menu for diet III was comprised of the foods ("high carbohydrate"):

Breakfast: Orange juice, bananas, milk, corn flakes with cream and sugar, toast, butter, jam, and coffee with cream and sugar.

Luncheon: Cream of tomato soup, crackers, hashed brown potatoes with a strip of bacon, bread, butter, jelly, peaches and cream.

Dinner: Escalloped potatoes, string beans, buttered beets, bread, butter, jam, ice cream, coffee with cream and sugar and two subjects had a small portion of cheese.

Each diet was preceded by a preliminary period of several days in order to determine the amount of food desired and to enable the subjects to establish a more or less definite system of routine. After the preliminary periods, no alterations were made in the diets, except during yeast periods when each subject ingested three or six yeast cakes daily. The results obtained during the period of the "high protein" diet (diet II) are given in Table I. Using the results obtained during the first control period for the basis of comparison, it is seen that the gas pressure increased in four and decreased in four of the trials when yeast had been added to the diet. After the first control period, there was practically no liquefaction of the egg albumen. At no time was

TABLE III

DIET III—"HIGH CARBOHYDRATE"*

		SUBJECT D.			SUBJECT H.			SUBJECT M.			SUBJECT P.		
PERIOD	DAY	GAS MM. HG.	LIQ. C.C.	NUMBER OF STOOLS	GAS MM. HG.	LIQ. C.C.	NUMBER OF STOOLS	GAS MM. HG.	LIQ. C.C.	NUMBER OF STOOLS	GAS MM. HG.	LIQ. C.C.	NUMBER OF STOOLS
	1	2	0	1	28	0	1	41	0	1	4	0	1
	2	8	0	1	7	0	1	12	0	1	41	0	1
First	3	17	0	1	6	0	2	3	0	1	17	0	1
Control	4	7	0	1	10	0	1	12	0	1	5	0	1
	5	9	0	1	7	0	2	3	0	1	0	0	1
	6	8	0	2	10	0	1	7	0	1	6	0	1
	Av.	9	0		12	0		13	0		12	0	
	1	6	0	1	3	0	1	8	0	1	20	0	1
Three	2	5	0	1	6	0	2	63	0	1	9	0	1
Yeast	3	6	0	3	13	0	3	37	0	1	No sample		
Cakes	4	0	0	0	2	0	2	52	0	1	16	0	2
Per	5	0	0	2	0	0	2	13	0	2	0	0	3
Day	6	4	0	1	5	0	2	19	0	2	8	0	2
	Av.	4	0		5	0		32	0		11	0	
	1	8	0	2	11	0	2	30	0	1	21	0	1
	2	10	0	1	0	0	2	29	0	1	18	0	1
Second	3	10	0	1	No sample			6	0	1	16	0	1
Control	4	7	0	2	16	0	2	40	0	1	62	0	1
	5	12	0	1	22	0	2	56	0	1	60	0	1
	6	8	0	1	9	0	1	25	0	1	27	0	1
	Av.	9	0		12	0		31	0		34	0	
	1	0	0	1	43	0	2	53	0	2	86	0	2
Six	2	4	0	1	24	0	1	39	0	1	60	0	2
Yeast	3	4	0	2	12	0	2	50	0	1	60	0	1
Cakes	4	2	0	1	5	0	1	11	0	1	21	0	1
Per	5	4	0	1	21	0	1	40	0	1	0	0	2
Day	6	2	0	1	21	0	1	31	0	1	17	0	1
	Av.	3	0		21	0		37	0		41	0	

*No corrections were made for changes in barometric pressure.

there liquefaction of the albumen to the extent reported by von Deschwanden. The reaction of the egg albumen whether solid or liquefied after twenty-four hours was always alkaline to litmus. Table II presents the chemical indices of putrefaction obtained during the course of diet II ("high protein"). The values represent averages of six-day periods. Subject D was constipated during the entire first control period and yet the gas pressure and liquefaction were less than in the cases of subjects M and P who were not constipated. It is of interest to note that the chemical indices of putrefaction were lowest with subject M, while the putrefactive indices calculated according to Rodella's method were higher than for any of the other three subjects. The putrefactive indices are not given in Tables I and III, because in the majority of experiments, there was no liquefaction of the albumen. Subject H, who had the highest chemical indices of putrefaction tended to have the lowest Rodella putrefactive indices.

The results obtained by the Rodella method during the "high carbohydrate" diet are presented in Table III and the chemical indices are given in Table IV.

TABLE IV

AVERAGE CHEMICAL INDICES OF PUTREFACTION FOR SIX-DAY PERIODS
DIET III—"HIGH CARBOHYDRATE"

PERIOD	SUBJECT D.			SUBJECT H.			SUBJECT M.			SUBJECT P.		
	TOTAL PHENOLS MG.	INDOLE MG.	INDICAN	TOTAL PHENOLS MG.	INDOLE MG.	INDICAN	TOTAL PHENOLS MG.	INDOLE MG.	INDICAN	TOTAL PHENOLS MG.	INDOLE MG.	INDICAN
First Control	614	4.5	+++	724	6.0	++	408	3.3	0	639	4.7	+
Three Yeast Cakes Per Day	601	6.2	++++	744	7.5	++	438	4.1	+	703	8.7	++
Second Control	608	5.3	++	677	8.5	++	421	2.5	+	591	4.2	+
Six Yeast Cakes Per Day	584	7.1	++++	707	9.4	++	441	3.9	++	600	8.1	+++

There was no liquefaction of albumen during this diet in any of the trials. The reaction of the albumen at the end of twenty-four hours was always alkaline. Using the results of the first control period for the basis of comparison, it is seen that gas pressure increased in four and decreased in four of the trials during periods of yeast ingestion. The chemical indices of putrefaction given in Table IV show that subject M had the lowest indices and yet the gas pressures tended to be as high or higher than those of subjects having the higher chemical indices of putrefaction. Subject H, whose chemical indices of putrefaction were the highest of the four subjects, had Rodella indices smaller than those of subjects M and P. Subject D, who was the only subject to experience constipation, had the lowest putrefactive indices obtained by the Rodella method.

It is difficult to understand why there was little or no liquefaction of the coagulated egg albumen after the first control period of diet II ("high protein") unless one assumes that there was a change in the flora of the intestine after yeast had been added to the diet. Rettger, Reddish and McAlpine,[3] however, found no change in the proportion of gram-positive and gram-nega-

tive organisms, or in the relative numbers of gram-positive rods of the acidophilus type as compared with all other intestinal bacteria when yeast was incorporated in the diet. Nevertheless, it seems possible that the flora may be changed, or at least the growth of certain members of the normal flora inhibited, for Gordon and McCleod[4] and Kilborn, Pierce and Tittsler[5] have shown that indole inhibits the growth of certain bacteria. The amount of indole eliminated during periods of yeast ingestion was larger than that eliminated during control periods. Again, indole production was increased by adding yeast to certain bacterial cultures, as will be shown in another paper.

It is recognized that chiefly the spore formers resist the temperature at which the egg albumen was coagulated. If these organisms were destroyed or if no spores were formed, few catabolic changes would be observed in the medium used. If the stools used contained few viable spore forming organisms, no marked chemical changes would take place, for these changes would be limited by the number of spores remaining in the albumen after heating.

As mentioned previously, the subject who was constipated had low Rodella putrefactive indices. This may be explained by the fact that the stools of constipated individuals contain fewer viable bacteria than soft stools which have not remained in the colon for periods of two or more days. However, there appears to be no relationship between gas formation and liquefaction and the number of stools passed daily.

It is likely that the type of diet used had some effect upon the flora, because there was no liquefaction of albumen during diet III, which was relatively high in carbohydrate. There may have been little or no spore formation and therefore little bacterial activity in the heated mixtures of albumen and feces.

The lack of agreement between the chemical indices of putrefaction and the Rodella putrefactive indices leads to the belief expressed by Bergeim,[6] namely, that more satisfactory chemical indices are needed for the measurement of intestinal putrefaction. The Rodella putrimeter as used in this investigation does not seem to offer a satisfactory means of determining the extent of intestinal putrefaction.

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P_H VALUES IN ROUTINE URANALYSIS*

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Los Angeles, Calif.

WE HAVE read with appreciation Myers and Muntwyler's[1] recent paper on urinary P_H. It has served to impress us once more with the wideness of the difference between the results obtainable by careful work and those that are ordinarily obtained by routine procedures. Of course, as these workers agree, a laboratory report of urinary P_H may differ from the correct value by as much as 0.2 or 0.3 of a unit and still be clinically useful; but surely the time is past when one who intelligently cares anything about the reaction of urine can be satisfied with a litmus paper test. It has been a bit surprising to find that as recently as 1926 this was the only urine reaction test mentioned in a well-known clinical laboratory manual,[2] and more surprising to learn by personal inquiry that it is the method still employed in the laboratories of a number of hospitals of considerable size.

Clark's[3] color charts used with suitable indicators provide measures of P_H that are accurate within 0.1 of a unit. Even LaMotte's[4] simplest outfit, using a single mixed indicator and ten color cards, allows for a maximum error of not much above 0.2 of a unit. Textbooks and manuals give plenty of advice about the details of uranalysis. It might seem as if every technician ought to get clinically dependable results without anything more being said on the subject of urinary P_H. Recent experience, however, in analyzing, or observing the analysis of, such urine specimens as compose a large share of those commonly sent by physicians to the clinical laboratories with which we have been in touch, convinces us that errors in reports of P_H may often be as much as a whole unit.

We fear that one group of physicians, while knowing that errors exist, may not realize their magnitude and may have more confidence in laboratory reports than they should. Another group, perhaps, better acquainted with the reports of research in uranalysis and rightly doubting that the degree of care used by research men will be exercised in routine clinical laboratory work, have come to undervalue the aid which they might get from this source. The data from some of the experimental work which we have done at times during the past two years, together with brief discussions, may lead the first group to a clearer idea as to which errors may be so great as to spoil the clinical usefulness of reports of urinary P_H and may show the second group how these errors can readily be reduced enough to make such reports of practical value. We hope to help narrow the gap between what investigators *know* and what routine workers *do*—not to announce the discovery of a new truth, but to make old truths more useful.

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In dealing with the error of approximately 0.2 of a unit, mentioned by Myers and Muntwyler[1] as due in large part to making the tests at room temperature instead of body temperature, one might follow the plan of subtracting this amount from all reported P_H values. It would be as satisfactory, however, to leave reports as they are and readjust one's idea of the normal average value, since routinely the tests are almost always made on specimens at room temperature.

From some work on diets which we have recently done in connection with another line of study, we find that the addition of moderate or large quantities of fruit or vegetables to the menu of a person whose urine is decidedly

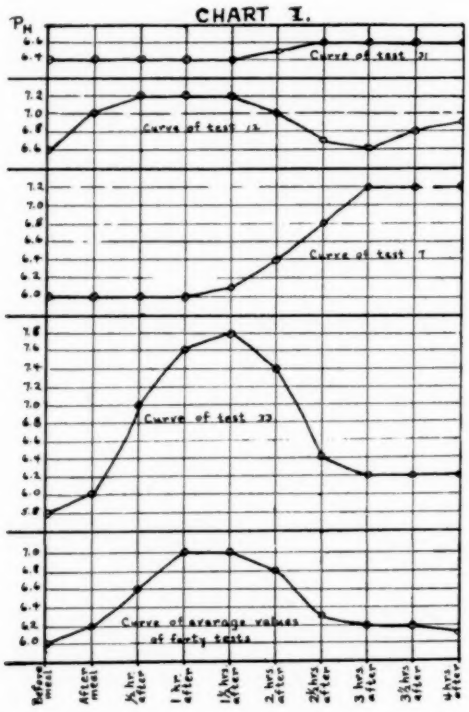

Chart 1.—Shows the variation in urinary P_H at different times of the day, with special reference to mealtimes and the "alkaline tide."

acid may make a difference of as much as 0.6 of unit in the P_H of two successive twenty-four-hour urine specimens, and that the taking of Rochelle salts may cause a similar change. These findings lead to the general conclusion that it would be well for the physician to direct that previous to and during the time of collection of a urine specimen the patient make no radical change in his diet or in the kind or the quantity of medicine that he may chance to be taking.

We have followed two lines of investigation to determine the difference that may be expected between reports based on tests of single as compared with twenty-four-hour specimens. First, twenty-four-hour specimens were collected from twenty different people, in each case a small part of the

"morning" voiding being reserved for a comparative test. In two cases the P_H of the single and twenty-four-hour specimens was found to be the same. In one case the single specimen was more acid than the twenty-four-hour specimen by 0.6 of a unit of P_H. An average of all the cases gave a difference of 0.23 of a unit, the "morning" specimens being more acid by this amount.

Second, in forty cases we determined the P_H of urine voided before a meal, after the same meal, and at half-hour intervals thereafter for four hours. On Chart 1 we give four curves that show various abnormalities and a fifth curve based on the average values obtained from the forty tests.

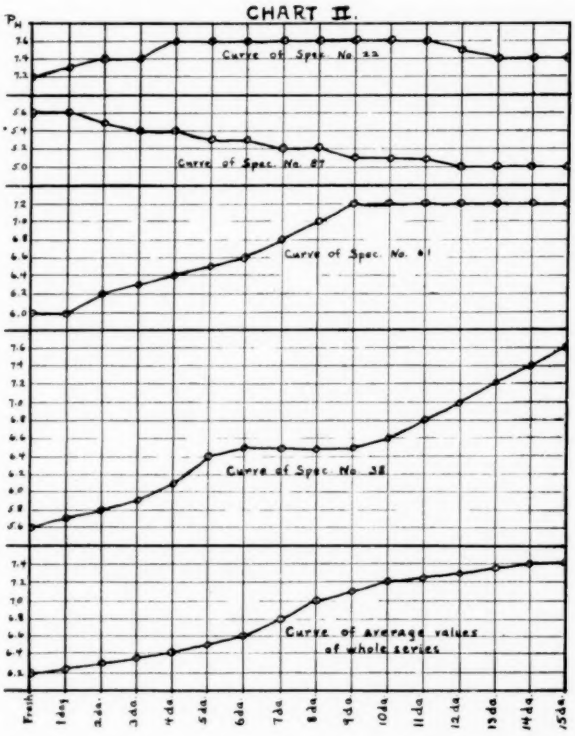

Chart 2.—Shows the changes observed in the P_H of urine that was let stand at room temperature without preservatives for fifteen days. The series was composed of nearly a hundred specimens.

From Chart 1 and the data which precede it one can see that the P_H of the urine as determined from a single voiding may differ from the average P_H for twenty-four hours by an amount so great as to be grossly misleading. Nothing short of insistence on twenty-four-hour specimens will do, if the physician hopes to get any useful information from reports of urinary P_H. Occasionally the special object of some intended test may call for a single specimen voided at some particular time, but this should be an exception to the general rule. It must be that the importance of this point is not understood as it should be, for we know that twenty-four-hour specimens are in a decided minority in many a laboratory.

Several of the common warnings or instructions about the details of uranalysis are based on the idea that the P_H of urine is unstable. It is true that changes take place if the urine is let stand at room temperature, especially if no preservative is used; but these changes do not take place so rapidly as some may fear. We have tested the P_H of nearly a hundred specimens, first when fresh and then daily for fifteen days, while they were kept without preservatives and at room temperature. On Chart 2 we give four curves that show abnormal changes and a fifth curve that shows the average change for the whole series.

While, as others[3] have pointed out and as is shown by Chart 2, an alkaline fermentation almost always occurs, in a few instances preceded by an acid fermentation, the change of P_H in twenty-four hours we find to be slight; and there is only occasionally as much as 0.2 or 0.3 of a unit's change in three days. Once a satisfactory specimen is obtained, therefore, it does not require any extraordinary speed nor the use of refrigeration or a preservative to secure a clinically significant report of its P_H value. It is not likely that the laboratory worker will delay the analysis long enough to cause any serious error from this source.

There are two types of errors that concern dilution. First, there is an actual change of P_H as the degree of dilution changes. Second, the color of the specimen itself, if dilution is not sufficient, may alter the shade of color given by the indicator and lead to unreliable results because of an apparent change of P_H. We believe that not enough attention has been paid to this second type of error, for in some instances we have found it to amount to as much as 0.4 of a unit.

Choosing very slightly colored specimens, so as to eliminate as far as possible the effect of the color of the specimens, we made over two hundred tests of the effect of dilution alone. In no case did changing the degree of dilution from 1-5 to 1-10 make as much as 0.1 of a unit's difference in P_H. In no case was the difference as great as 0.2 of a unit when the dilution was changed from 1-5 to 1-20. Occasionally, changing the dilution from 1-5 to 1-15 made 0.1 of a unit's difference.

Aside from methyl red, which need not be used, most of the indicators employed in uranalysis are yellow on the acid side.[3] Since the commonest color of urine is also some shade of yellow, the tendency, especially through the middle and upper parts of the color ranges of these indicators, is for the apparent P_H of the urine-indicator mixture to be shifted toward the acid side. The shift due to dilution, on the contrary, is toward the alkaline side, except when one is dealing with neutral or alkaline specimens, which are much less numerous than acid specimens and in which the degree of dilution seldom makes much difference in P_H, since the reaction is at or near the neutral point. We have, then, in the majority of cases, the fortunate condition of errors from one source more or less offsetting those from another.

Ordinarily, any convenient degree of dilution, such as 1-5 or 1-10, will give satisfactory results. With deeply colored specimens, dilutions as great as 1-15 or 1-20 may be needed to strike a balance between the errors of dilution and specimen colors. In any case, however, since moderate changes in

the degree of dilution affect the P_H so slightly, time spent in accurately measuring volumes of urine and diluent is wasted, so far as the clinical value of the results is concerned. The laboratory worker will do well as a rule to make approximately 1-5 dilutions of slightly colored specimens and increase the degree of dilution as the depth of color of the specimen increases until a maximum of approximately 1-20 is reached. This rule will materially decrease the number of errors that amount to as much as 0.2 of a unit.

SUMMARY

From the research point of view, carefulness in measurement is essential. In routine work there is seldom time to follow out all the details of technic necessary to test urinary P_H with real accuracy; but, if tests of P_H are to be made at all, enough care must surely be exercised to prevent reports being so erroneous as to be worthless to the physician. Many errors are, clinically speaking, negligible; but our experimental data show that attention is especially needed to the following points, if errors are to be kept within clinically allowable limits:

1. Patients should be told not to make radical changes in diet or medication before or during specimen collection periods.

2. Reports based on single specimens may be grossly misleading. The physician should insist on twenty-four-hour specimens.

3. In testing for urinary P_H, deeply colored specimens should be diluted more than those that are slightly colored.

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A UNIFORM SYSTEM OF HEMATOLOGIC METHODS FOR USE WITH OXALATED VENOUS BLOOD*

By Edwin E. Osgood, M.D., Howard D. Haskins, M.D., and Frank E. Trotman, M.D., Portland, Ore.

THE advantages in convenience, speed, and accuracy of using oxalated venous blood instead of capillary blood from the finger or ear, for red cell counts, cell volume determinations, and hemoglobin estimations became so obvious to us during an extensive study of normal values for color, volume, and saturation index standards,[1, 2] that we decided to use it routinely in the hospital and dispensary laboratories under our control and to determine what other hematologic methods could be done satisfactorily on oxalated blood. It is the results of these studies which we here present, and in the articles[3, 4] immediately following, we give the details of the technic for some of the methods that we recommend for use on oxalated blood.

METHODS† WHICH MAY BE DONE ON OXALATED VENOUS BLOOD

We have determined that oxalated venous blood is quite suitable for the following methods: hemoglobin estimation (twenty-four hours), red cell count (twenty-four hours), platelet count (one hour), red cell volume (three hours), color index (twenty-four hours), volume index (three hours), saturation index (three hours), icterus index (four hours), van den Bergh test (four hours), white cell count (twenty-four hours), making the smear for differential count (one hour), peroxidase test (three hours), fragility test (three hours), and sedimentation rate (three hours). The time indicated in parenthesis is the period within which the estimation must be performed in order to insure accuracy. The blood preferably should be kept in the ice box; this is quite necessary if the blood is to stand over three hours.

ADVANTAGES OF USING OXALATED BLOOD

Convenience.—The greater convenience[5, 6] of this method is shown by the following points: (1) Only a syringe, sterile needle, tourniquet, test tube

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This work was awarded the Bronze Medal in Class I when presented in the Scientific Exhibit of the American Medical Association in July, 1929.

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†It is probable that still other methods can be done to advantage on oxalated venous blood. We have demonstrated reticulocytes in blood which has stood three hours after the addition of oxalate but have not a sufficiently large series to state definitely whether the percentage count is unaltered. Spohr[18] reports that oxalated blood is satisfactory for the reticulocyte count. Preliminary studies suggest that plasma and cells from oxalated blood are as satisfactory as serum and cells obtained in the usual way for blood grouping. As yet uncompleted studies being made at our suggestion in the Department of Bacteriology in this school have not yet shown any interference of the oxalate in such serologic procedures as the Wassermann, Kahn, and commonly done agglutination reactions. Details of this work will be reported at a later date in papers from this department.

containing oxalate, cotton, and alcohol need be carried to the patient. (2) The estimations do not have to be made immediately, and if there is a suspicion of error, they can be repeated without the embarrassment of returning to the patient for more blood. (3) If blood is desired for a Wassermann, coagulation time, or for blood chemistry tests, no extra puncture is necessary. (4) If during the study of the blood, further hematologic work is deemed desirable, it may be done on the same sample. (5) If an unusual or interesting blood picture is encountered, as many slides as are desirable can be made for future reference or for teaching purposes, without again disturbing the patient. (6) If the smear is made and sent along for staining and examination, blood can be mailed considerable distances to a central laboratory for expert examination.

Accuracy.—The greater accuracy[5, 6] is shown by the following points: (1) Larger quantities of blood may be used for the hemoglobin estimation, insuring more accurate results. (2) Time is allowed for making measurements. The haste necessary to prevent clotting (cell counts, fragility test, etc.) when capillary blood is used predisposes to inaccuracy. (3) Errors (which are avoidable, but seldom avoided) due to manipulation or constriction of the part, to local edema, or congestion common to the use of capillary blood are completely done away with. Drucker[7] has shown that in order to get accurate results on capillary blood, a sufficiently deep wound must be made to cause at least three drops of blood to flow without any manipulation whatsoever, and that the first two drops must be discarded. Any one who has tried to train medical students or technicians knows how few of them actually observe these essentials for accurate counting. If these precautions are observed, counts agreeing with those on venous blood are obtained.

(4) Duplicate estimations can be run on the same sample which serves to increase accuracy, show up poor technic and poor methods, and allow one to form an opinion of the accuracy being attained. If the work is done by technicians, questionable results can be checked at any time on the same sample of blood. By having them occasionally run a series of estimations on the same sample, they learn their experimental error and can be encouraged to reduce it. New technicians or students can be taught to repeat results on the same sample until they consistently agree within the desired limits. Hematologic work can be sent to a central laboratory and its accuracy can be checked occasionally by dividing a well-mixed sample between two tubes and either sending in one and examining the other or sending in both tubes with different labels and noting how the results agree.

Further advantages.—These are: (1) Normal values[1, 2, 5, 6] have been more carefully standardized on venous blood, and many who have compared it with capillary blood prefer it. (2) It is less disagreeable to the patient. Venipuncture is less painful than puncture of the ear or finger.[8] Twenty of twenty-eight students who were asked, expressed a preference for venipuncture to finger or ear puncture. (3) One puncture suffices for Wassermann, hematology, blood chemistry, etc.

ANSWERS TO OBJECTIONS TO THE USE OF VENOUS BLOOD

1. Venipuncture is not practical on all patients. This is valid, and the older methods should be used on certain children and obese patients with small veins.

2. The quantity of blood removed will harm the patient. This is a myth. If 5 c.c. of blood were withdrawn daily for ten days, and no regeneration occurred, but the blood volume returned to its previous value, the hemoglobin percentage would theoretically be about one per cent lower than on the first day. Normal daily variations far exceed this.

3. Patients object to it. This is very rare. The method has been in routine use in a 250 bed hospital for three years with satisfaction to all concerned. Patients' objections to venipuncture have not prevented the steady increase in the number of hospitals and clinics in which a blood Wassermann is a routine procedure.

4. Finger-blood or ear-blood with micro-methods is just as good. We and others have conclusively shown that this is untrue. Has not the patient with pernicious anemia or other serious anemias the same right to an early and accurate diagnosis as has the patient with diabetes mellitus or nephritis on whom we do repeated venipunctures for blood chemistry?

METHODS AND PRECAUTIONS FOR USING VENOUS BLOOD

1. Prepare a month's supply of test tubes, containing 2 mg. of dry potassium oxalate per c.c. of blood to be taken, by measuring into each, with a burette, 0.1 c.c. of 2 per cent potassium oxalate for each c.c. of blood to be taken and evaporating this to dryness in a drying oven.* Keep the tubes corked and in baskets labelled with the amount of blood for which they were prepared (3, 5, 10, 15, and 20 c.c. is a convenient distribution). Three c.c. of blood is sufficient for a routine hematologic examination and 10 c.c. for a complete examination.

2. Draw blood from the vein by the usual technic, but the tourniquet should be released if more than two minutes are required for securing sufficient blood, as we have found that the red cell count begins to increase after three minutes of stasis.

3. Remove the needle from the syringe before running the blood into the oxalate tube as hemolysis will result if the blood is forced through the needle.

4. Cork the test tube (never use cotton) and at once shake vigorously by holding the test tube horizontally in the left hand and tapping the other end with the right hand.

5. The blood must be thoroughly mixed in this manner for thirty seconds immediately before samples are withdrawn for any test.

6. Samples should be taken directly from the test tube, not from blood poured out on a slide or watch glass.

7. The tube must be kept corked at all times when not in use.

*For small laboratories, 1 large drop (0.07 c.c.) of saturated potassium oxalate (30 per cent) is sufficient for 10 c.c. of blood and has a negligible dilution effect.

8. The time limits noted should be observed if the most accurate results are desired, although as a rule a slightly longer time will not introduce clinical error.

HEMOGLOBIN ESTIMATION

Any technic may be used. We prefer the Haskins-Sahli[9] method when few estimations per day are made, and the modified Osgood-Haskins[3] method for larger laboratories.

RED CELL COUNT*

We prefer to use Toisson's diluting fluid. The details of the technic are the same as for any accurate red cell count, but thorough mixing of the blood immediately before withdrawing it from the test tube is especially important. The most frequent clinical errors in red cell counting are the use of absorbent material to wipe off the tip of the pipette and failure to wait three minutes to allow settling of the cells on the ruled area before beginning the enumeration.

PLATELET COUNT

If oxalated blood and freshly filtered Toisson's diluting fluid are used, the platelet count can be done on the same preparation used for the red cell count (1 in 200 dilution). The counting chamber should be allowed to stand ten minutes before beginning the enumeration. The platelets appear under the 4 mm. objective as highly refractile dots, 1 to 4 micra in diameter. We count all the platelets over 1 sq. mm. (one-ninth of the ruled area). Rather than count 1 corner square, we count a diagonal group of 4 large squares from each of the 4 corner groups of 16 large squares (ordinarily studied in the white cell count) as this gives a better average of the total distribution in the chamber. The total number observed multiplied by 2000 (10×200) gives the number per cu. mm. of blood.

RED CELL VOLUME

Our technic has been published.[1, 2, 10] Narrow centrifuge tubes prepared according to our specifications are now available.†

COLOR, VOLUME AND SATURATION INDEXES

These should be calculated on the basis of the recently determined normal standards.[1, 2, 5, 6] A table and chart greatly simplifying these calculations has been published.[10]

ICTERUS INDEX

We prefer the technic recommended by Bernheim,[11] but any method will give results as good as those on serum if similar precautions are observed to take the blood in the morning before breakfast (avoids postprandial lipemia), to rule out carotinemia, and to discard samples showing hemolysis. Accord-

*Complete details of all these methods will be found in the textbook mentioned in the last footnote on the first page of this article.

†These may be obtained from the Arthur H. Thomas Co., West Washington Square, Philadelphia.

ing to our experience, there is no greater tendency to hemolysis in oxalated blood than in serum if the following conditions are met: use a dry syringe and needle; remove the needle from the syringe before ejecting the blood; and centrifugate within four hours after the blood is withdrawn.

THE VAN DEN BERGH TEST

The results on oxalated blood are similar to those on serum.

WHITE AND DIFFERENTIAL CELL COUNT

The technic is identical with that on capillary blood. The sooner the smear is made for the differential count, the better the results, but gross inaccuracies are rarely encountered if the smear is made within three hours after taking the blood. If through carelessness, a great excess of oxalate is used, the nuclei of the polymorphonuclears may lose their lobulated form and become rounded. Smears may be stained at any time within twenty-four hours. A buffer phosphate solution* (P_H 6.4), as recommended by McJunkin,[12] gives better results than distilled water for Wright's stain.

PEROXIDASE TEST

We prefer the method of Washburn[13] but we use buffer phosphate* instead of distilled water with the Wright's stain.

FRAGILITY TEST

Any technic may be used. We prefer the Giffin and Sanford[14] modification of the Ribierre method, but take 0.1 c.c. as a unit rather than 1 drop, as this makes possible much more rapid measurement. The use of oxalate is a great convenience in this test because it is not necessary to carry tubes to the patient's bedside, or to use great haste (to avoid clotting) in delivering the drops of blood into the tubes. Freezing point determinations on plasma indicate that the quantity of oxalate used is equivalent to an increase of only 0.0055 per cent in concentration of sodium chloride solution in each tube. Comparative studies on the same blood with and without oxalate show no detectable differences in the readings.

SEDIMENTATION RATE

This method is given in a separate article.[4]

SUMMARY

Fourteen of the hematologic methods most commonly done have been adapted for use with oxalated venous blood.

The use of this system of methods is a great convenience to the patient, the clinician, and the technician.

In certain of the methods, the use of oxalated blood favors greater accuracy.

*The buffer phosphate solution may be prepared by dissolving 6.63 gm. of Merck's monopotassium phosphate and 2.56 gm. of Merck's anhydrous disodium phosphate in 1 liter of distilled water (add about 1 c.c. of chloroform).

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A SIMPLIFICATION OF THE OSGOOD-HASKINS HEMOGLOBIN METHOD*

By Edwin E. Osgood, M.D., Howard D. Haskins, M.D., and Frank T. Trotman, M.D., Portland, Ore.

THIS is one of the group of methods included in our uniform system of hematologic methods designed for use with oxalated venous blood.[1]

The ideal hemoglobin method should be accurate, rapid, simple, and require no expensive apparatus or reagents not used for other purposes. The method here described meets these requirements for laboratories which own a colorimeter and perform ten or more hemoglobin estimations per day. Smaller laboratories will find Haskins' modification [2, 3] of the Sahli method more satisfactory.

The original method[4] is an acid hematin method, using an ordinary colorimeter and a permanent standard† which contains only inorganic salts and has not changed in seven years. It is best suited for use with oxalated venous bood, although blood from the finger or ear can be used.

It is performed as follows: Measure exactly 1 c.c. of *well mixed* oxalated blood into a 100 c.c. volumetric flask‡ containing about 40 c.c. of distilled water.§ After laking has occurred, add about 50 c.c. of N/5 hydrochloric acid§ while mixing, and dilute to the mark. If there is foam, cut it with a drop of ethyl alcohol (do not use caprylic alcohol). Mix thoroughly and pour part of the solution into a test tube. Heat the tube in a bath at 55 to 60° C. for seven minutes (or longer), and cool. Estimate with a colorimeter, setting the permanent standard at 15 mm. When 3 or 4 readings have been made, take the temperature of the standard at once by inserting a clean dry thermometer in the liquid in the colorimeter cup.

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†The permanent standard contains 32 grams of ferric sulphate and 80 mg. of chromic sulphate in 100 c.c. of solution. It cannot be produced by weighing out the constituents and dissolving, since ferric sulphate of uniform color value is not obtainable. Standard solution (checked by Dr. E. E. Osgood) can be secured from Hynson, Westcott and Dunning, Baltimore, or from the Shaw Supply Co., Portland, Oregon.

‡If less blood is available, dilute 0.5 c.c. in a 50 c.c. flask. In marked anemia, dilute 1 c.c. of blood to 50 or 25 c.c. and divide the calculated results by 2 or 4, respectively. If finger or ear blood is used, measure exactly 0.05 c.c. into exactly 2.45 c.c. of water (previously measured into each of a series of test tubes which are kept tightly corked), rinsing the pipette as in the Sahli method; when laked, add exactly 2.5 c.c. of N/5 hydrochloric acid.

§The use of two overhead siphon bottles of 2 liter capacity to contain distilled water and N/5 hydrochloric acid speeds up the measurements. A 5 c.c. variation is unimportant (the N/5 hydrochloric acid may be 45 to 55 c.c.). A measuring device may be inserted into the siphon tract: cut the bottom off an 8 x 1 inch test tube and fit with stoppers, a two-hole stopper at the top connects with the siphon and with an air vent tube; a one-hole stopper at the bottom connects with a glass tube (and rubber tube) for delivery. There must be a pinchcock above to control inflow and another below for outflow. Graduate one for 40 c.c. (and 20) and the other for 50 c.c. (and 25) by measuring water into the tube and making marks with a file or grease pencil.

Calculation.—In the tables furnished with the standard (see Table I for excerpt) read the percentage in the proper temperature column opposite the average mm. reading,* interpolating when necessary. Convert the per cent figure into grams per 100 c.c. of blood by using the combined table for hematologic calculation.[5]

Instead of being heated, the acid hematin solutions may stand at room temperature twenty-four hours, or even several days, before being estimated. They must be shaken well before filling the colorimeter cup (acid hematin "solution" is a suspension).

TABLE I

A PORTION OF THE TABLE* FOR DETERMINING PERCENTAGE HEMOGLOBIN USING OSGOOD AND HASKINS' PERMANENT STANDARD

COLORIMETER READING MM.	TEMPERATURE OF THE STANDARD										
	15.5°	16.5°	17.5°	18.5°	19.5°	20.5°	21.5°	22.5°	23.5°	24.5°	25.5°
10.0	105.0	107.1	109.2	111.5	113.5	115.8	118.2	120.5	123.1	125.6	128.2
10.1	104.0	106.0	108.1	110.2	112.3	114.5	116.8	119.3	121.8	124.3	126.9
10.2	103.0	105.0	107.0	109.1	111.2	113.5	115.7	118.1	120.5	123.1	125.7
10.3	102.0	104.0	106.0	108.0	110.1	112.2	114.3	116.7	119.2	121.8	124.4
10.4	101.0	103.0	105.0	107.0	109.0	111.1	113.2	115.6	118.0	120.6	123.2
10.5	100.0	102.0	104.0	106.0	108.0	110.0	112.0	114.5	117.0	119.5	121.9
10.6	99.0	101.1	103.0	105.0	107.0	109.0	111.0	113.5	116.0	118.4	120.8
10.7	98.1	100.2	102.2	104.1	106.1	108.2	110.3	112.6	115.0	117.3	119.7
10.8	97.4	99.4	101.4	103 3	105.2	107.2	109.3	111.6	113.9	116.2	118.7
10.9	96.6	98.5	100.4	102.2	104.1	106.1	108.1	110.4	112.7	115.1	117.6
11.0	95.8	97.6	99.4	101.2	103.0	105.0	107.0	109.2	111.5	114.0	116.5
11.1	94.9	96.7	98.5	100.3	102.1	104.1	106.2	108.4	110.6	113.9	115.4
11.2	94.0	95.8	97.6	99.4	101.2	103.2	105.2	107.2	109.7	112.0	114.4
11.3	93.1	95.0	96.8	98.6	100.4	102.4	104.5	106.6	108.8	111.0	113.4
11.4	92.2	94.1	96.0	97.8	99.6	101.5	103.3	105.5	107.9	110.1	112.5
11.5	91.3	93.2	95.1	96.9	98.8	100.5	102.5	104.7	106.9	109.1	111.5
11.6	90.7	92.5	94.3	96.1	98.0	99.9	101.7	103.8	106.0	108.2	110.5
11.7	89.9	91.7	93.5	95.3	97.1	98.9	100.8	102.9	105.0	107.0	109.0
11.8	89.2	90.9	92.6	94.4	96.2	97.9	100.0	102.0	104.1	106.3	108.6
11.9	88.5	90.2	91.9	93.6	95.4	97.3	99.2	101.2	103.3	105.4	107.5
12.0	87.8	89.5	91.2	92.9	94.7	96.5	98.4	100.4	102.5	104.6	106.8

*The complete tables (furnished with the standard) extend over colorimetric readings from 8.0 to 20.4 mm. and temperature variations in the standard from 15.5 to 31.5° C. The results are in per cent, calculated on a basis of 13.8 grams as 100 per cent. Tables for conversion of the percentage figure to grams per 100 c.c. are given in reference 5.

Preservation of the standard. Keep it in a Pyrex flask, corked tightly with a rubber stopper (if a new one is used, remove the powdered material adhering to it). The standard, after being used, is stored in a second flask. Before the main stock is exhausted, check up the used standard against it by making estimations with each, using the same acid hematin preparation. If they agree, pour the used standard into the other flask and use it again. If the used standard has become contaminated, it will usually be off color as well as give incorrect readings. If it seems to be incorrect, filter it. Recheck the setting of the colorimeter, and then check the filtrate before deciding to discard it. We have found that, with reasonable care, no change in the solution is noticed during months (or years) of repeated use.

*If the colorimeter reading is higher than 20.4 mm., divide it by 2 and look up the per cent in the table, and then divide by 2. A better method is to make a fresh preparation of acid hematin, diluting to 50 or 25 c.c. instead of to 100 c.c.; the calculated result is then divided by 2 or 4.

Apparatus.—Accurate 1 c.c. pipettes and volumetric flasks, colorimeter, a dropping bottle (for the alcohol), siphon bottles, and permanent standard. A 56° C. water-bath is desirable.

Reagents.—N/5 hydrochloric acid (18 c.c. of c.p. hydrochloric acid diluted to 1 liter) and ethyl alcohol.

An error of over 2 per cent is unusual in routine work and, with research care, it can be held within 1 per cent. While this method has been used with satisfaction in many large clinics and hospitals and has made possible an extended research on hemoglobin values in health[6, 7] and disease,[8] it has certain disadvantages. These are the possibility of contamination of the standard through carelessness in cleaning of colorimeter cups, plungers, or thermometer, and the extra time required to take the temperature of the standard after each colorimeter reading. These disadvantages are obviated to a large extent by the modification proposed.

Technic of the Modified Osgood-Haskins Method.—Make a careful estimation by the original Osgood-Haskins method on one of the bloods obtained at the first of each week. Heat to develop the full color and keep the entire 100 c.c. sample of acid hematin in a brown bottle (preferably in the ice box) labelled with the percentage of hemoglobin found, the temperature of the permanent standard, and the colorimeter reading corresponding to it (e.g., 111.5 per cent at 11.5 mm. and 25.5° C.).*

For the rest of the week, use this as the standard (shake to secure uniform suspension before using), placing it in the standard cup and setting at the same reading as that noted on the bottle (e.g., 11.5 mm.). The temperature of the standard is not taken, since the color of the acid hematin does not vary.

Calculation.—Use the same table as for the original method, finding the percentage hemoglobin opposite the average mm. reading in the same temperature column as that marked on the bottle containing the acid hematin standard (e.g., 20.5° C.).

By this modified method, much time is saved and one batch (50 c.c.) of permanent standard can be kept in good condition for a year or more, no matter how many estimations are run. Our technicians require less than 2 minutes per estimation.

The apparatus and reagents are the same as for the original method. It is desirable to have the pipettes graduated to *contain* 1 c.c. to the tip, for each can then be stood up in the 100 c.c. flasks to drain while the next pipette is being filled and the water from the siphon bottle is then delivered through each into the flask, thus rinsing out the remaining blood and insuring accurate measurements without the necessity of care in observance of the rate of flow and drainage time.

In Table II are shown comparative results by the original technic and the modified method. Research care was used in the estimations by the original technic. As previously shown,[4] these results will not deviate by more

*If the reading obtained involves interpolation, e.g., 100.5 per cent at 10.55 m.m. and 16° C., find the percentage elsewhere in the table, e.g., 100.5 per cent at 11.5 mm. and 20.5° C., and note these figures on the bottle.

TABLE II

COMPARISON OF THE ORIGINAL AND THE MODIFIED METHODS

ACID HEMATIN STANDARD USED	BLOOD NO.	HEMOGLOBIN				DIFFERENCE PER CENT
		INORGANIC STANDARD		ACID HEMATIN STANDARD		
		PER CENT	GRAMS	PER CENT	GRAMS	
A	6	71.7	9.90	69.2	9.55	−3.53
A	7	84.6	11.67	79.8	11.01	−5.65
A	8	88.0	12.14	89.2	12.31	+1.40
A	9	85.5	11.80	82.2	11.35	−3.81
B	10	109.9	15.16	109.1	15.05	−0.73
B	11	92.3	12.74	91.8	12.67	−0.55
B	12	101.0	13.94	102.3	14.12	+1.29
B	13	99.6	13.74	97.8	13.50	−1.75
B	14	127.1	17.54	128.2	17.69	+0.86
B	15	91.7	12.66	88.6	12.22	−3.48
C	21	117.4	16.21	123.8	17.08	+5.37
C	22	96.0	13.25	98.8	13.63	+2.87
C	23	119.9	16.54	121.5	16.77	+1.39
C	24	115.8	15.98	115.5	15.94	−0.25
C	25	106.9	14.75	108.1	14.91	+1.08
D	36	107.0	14.77	105.2	14.52	−1.69
D	37	94.2	13.00	94.2	13.00	0.00
D	38	93.5	12.90	93.7	12.93	+0.23
D	39	127.5	17.60	128.2	17.69	+0.51
D	40	112.3	15.50	112.8	15.57	+0.45
E	42	113.4	15.65	115.7	15.97	+2.04
E	43	116.5	16.08	115.7	15.97	−0.68
E	44	128.9	17.78	131.0	18.08	+1.69
E	45	108.6	14.98	109.8	15.15	+1.13
E	46	106.8	14.74	108.7	15.00	+1.76
Average		104.6	14.43	104.8	14.46	+0.19

than plus or minus 2 per cent from the average of a series of hemoglobin determinations by the van Slyke oxygen capacity method. The results by the modified technic are those secured by the technician in routine work. Twenty-five estimations made with five different acid hematin standards were selected as typical of the larger series of forty-six comparisons made. They include the greatest plus and minus deviations noted in the larger series. All of the estimations made with one acid hematin standard are consecutive estimations made on the same day but not necessarily on the day on which the standard was made up. In the larger series, no greater deviations from the correct figure were observed on the last day of use of the acid hematin standard than on the first day. Note that the greatest deviations observed in the series of forty-six consecutive comparisons are plus 5.37 per cent and minus 5.65 per cent and that 90 per cent of the results show a deviation from those by the other technic of less than plus or minus 4 per cent. The probable error calculated from this series is plus or minus 1.58 per cent.

The method has been in use in a two hundred and fifty bed hospital for over two years with satisfaction to all concerned. It has all the advantages of the methods of Cohen and Smith[9] and of Robscheit,[10] with the following additional advantages: The standard requires only five minutes of the technician's time to prepare and no apparatus not used in the actual estimation, except a thermometer. The full color of the acid hematin is developed. The acid hematin standard is discarded at the end of one week instead of after several months, thus greatly decreasing the likelihood of any change in color value.

We know of no other method which permits estimations of equal speed and accuracy except the photoelectric hemoglobin method of Sheard,[11] and this requires expensive apparatus, precluding its use in any but the largest laboratories.

SUMMARY

A hemoglobin method is presented which has limits of error within plus or minus 6 per cent in routine work. One estimation requires two minutes or less to perform, if several are done in one day. No expensive reagents or apparatus are required other than a colorimeter such as is found in most clinical laboratories.

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A RAPID METHOD FOR DETERMINATION OF THE SEDIMENTATION RATE OF THE RED CELLS WITH RESULTS IN HEALTH AND DISEASE*

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THE determination of the rate of sedimentation of the red cells of the blood has been shown to have considerable clinical value. It seemed to us desirable to have a method of estimation using oxalated blood that was simple, rapid, and consuming as little time as possible.

As a result of our work, we propose a modification of Westergren's method. We have tested the blood of almost 2000 individuals including almost 300 normal young adults, using this method. This is one of the group of methods included in our uniform system of hematologic methods for use with oxalated venous blood[1]

All of the methods in use have certain disadvantages. All require an extra manipulation, namely, dilution with sodium citrate solution.

The method (Linzenmeier's) which is probably most generally used in this country, is the most objectionable. The blood must be watched closely until the cells reach the 18 mm. mark on the tube, a very unreasonable specification. The small test tubes (inside diameter, 5 mm.) that are used are difficult to clean.

Westergren's method seems to be extensively used in Europe. It is a good method. As ordinarily carried out, the first reading is made after one hour settling of the blood, and some investigators make only the one reading. This is fairly satisfactory for normal and other slow-settling bloods. Our work shows that in the case of bloods sedimenting at a faster rate, the first reading must be made within fifteen minutes after starting the test, in order to get a true idea of the rapidity of settling. In the case of the fast-settling bloods, the final reading must be made within *less* than an hour (e.g., forty-five minutes).

Methods that call for readings every five or ten minutes for an hour or more, are too time-consuming to be adapted to routine clinical use, although the results secured are very interesting from a research standpoint. The plotting of a curve for each sedimentation test is not of clinical significance. Curves actually secured do not conform even approximately to the four model curves proposed. See Fig. 1 for sedimentation rates for fifteen-minute periods secured by us in one type of case (uncomplicated salpingitis), and notice that all intermediate points between the model curves of the graphic method are filled in solidly (many other results were secured which could not be drawn in the figure).

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The Proposed Method.—We use the same kind of pipette* as Westergren, but do not dilute with citrate solution, and we finish the test fifteen minutes before the first reading is made by the Westergren method.

As anticoagulant we use dry potassium oxalate (2 mg. per c.c. of blood) as described in another paper.[1] Thus, we avoid dilution of the blood and an extra manipulation, and use a portion of the same blood as for blood chemistry and hematologic work. Comparative studies of oxalated blood and blood diluted with citrate solution showed that the use of oxalate did not introduce clinical error. We have determined the sedimentation rates for

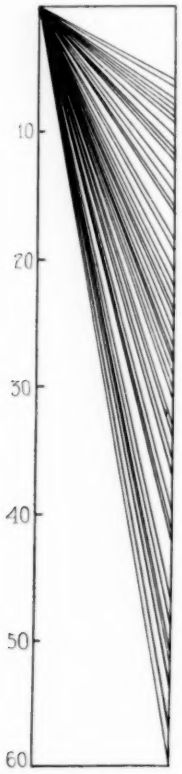

Fig. 1.—Sedimentation in uncomplicated salpingitis (mm. maximum settling in fifteen minutes).

normal individuals using blood oxalated as recommended. The test is to be started within three hours after drawing the blood.

Technic.—Fill the pipette to the zero mark with *well-mixed* venous blood, wipe off the excess blood and place in the sedimentation rack† (see Fig. 2). Be sure to press the tip of the pipette firmly against the lower rubber support before releasing the finger, and place at once under the spring that supports the top of the pipette. Note the time, also read the level of the blood if it is not exactly at zero.

*The pipette is 300 mm. long and is graduated in millimeters from 200 at the tip to zero above. It contains about 1 c.c. at the zero mark. These pipettes are obtainable from Arthur H. Thomas and Co., Philadelphia (B. P. specifications No. 1710 C).

†Obtainable from the National Appliance Co., 211 Oak St., Portland, Ore.

After fifteen and forty-five minutes from the start, make readings of the upper level of the red cells; note both readings on the clinical record. If the cells have not settled sharply, make a rough estimate of the approximate level.

The advantages of this method are obvious to those who are familiar with the current methods; we can summarize them briefly: the results are secured quickly (within forty-five minutes); very little manipulation is required and there is little expenditure of a technician's time; the same blood as for other

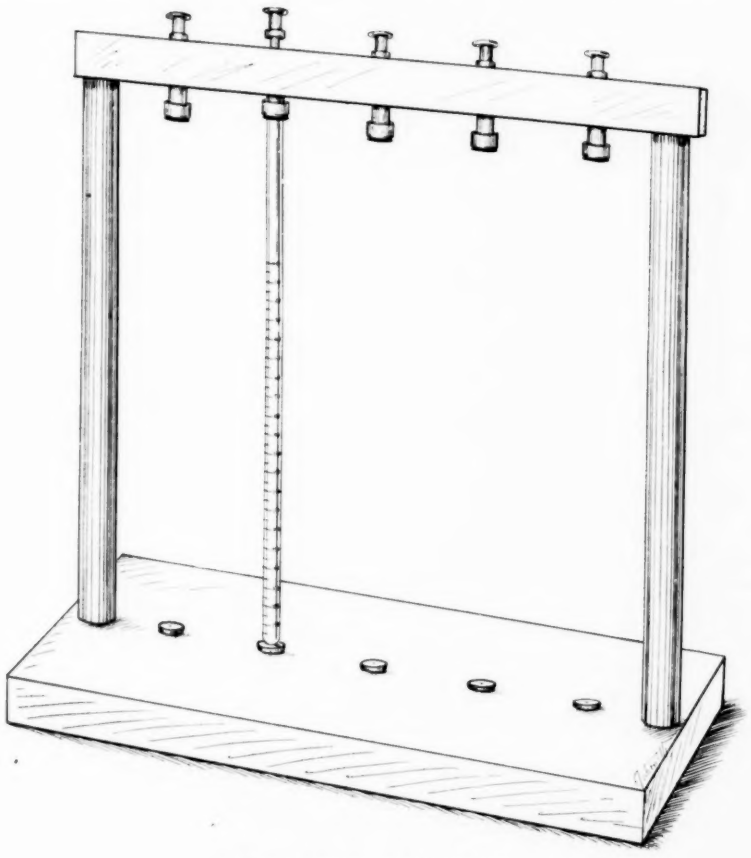

Fig. 2.—Sedimentation rack.

laboratory methods is used; and the sedimentation rate determination by this method is closer to the true rate than by most methods.

Calculation of the Rate.—Preliminary work led us to choose the settling in a thirty minute period (occurring between the fifteen and the forty-five minute readings) as expressing the rate, and this was used in our first paper.[2]

After studying the results of a very large number of tests, we concluded that the *maximum* settling in a fifteen minute period expresses the rate more satisfactorily. Find the rate as follows: deduct the reading for the fifteen minutes settling from that for forty-five minutes settling, and divide by two;

now compare this figure with the millimeters settling during the first fifteen minutes. The larger figure (maximum settling in fifteen minutes) is the sedimentation rate.

There are conditions that tend to interfere with uniform settling so that the maximum rate of settling observed is necessarily nearest to the true rate for a particular blood. Most bloods show a distinct lag in the speed of settling during the first fifteen minutes; these show a more uniform rate of settling in the thirty minute period following the fifteen minute reading. Bloods that settle very fast are slowed up during part of the thirty minute period because the cells are approaching a packed condition. A few bloods show a uniform rate of settling throughout the forty-five minutes of the test.

A rate per minute might be used, but would be unsatisfactory because a large number of results would be decimal fractions, e.g., a high normal rate would be 0.33 mm.

It is advisable to note on the clinical record the millimeter settling in fifteen and in forty-five minutes, as well as the rate that is calculated from these readings. When one becomes expert in interpreting sedimentation data, he will sense the rapidity of settling instantly from the two readings without stopping to calculate the rate.

SUMMARY OF SEDIMENTATION TESTS MADE

Normal individuals	295	295 estimations
Obstetric cases[2]	305	537 estimations
Gynecologic[2] cases	620	1344 estimations
Medical and surgical cases	605	708 estimations
Total cases	1825	2884 estimations

Sedimentation Rates of Normal Individuals.—All our normals were healthy young adults, chiefly nurses and medical students.

Practically all normal adults have a maximum sedimentation in fifteen minutes of less than 5 mm. (see Table I). In 295 cases only 5 per cent were above that figure. Some may have had a focus of infection that was not recognized.

TABLE I

SEDIMENTATION RESULTS IN NORMAL PERSONS

	NO. OF CASES	AVERAGE 15 MIN. READING MM.	AVERAGE 45 MIN. READING MM.	AVERAGE RATE MM.
Females:				
Group 1	104	0.6	2.5	1.0
Group 2	96	1.6	6.4	2.6
Group 3	14	3.0	18.0	8.0
All	214	1.0	5.2	2.2
Males:				
Group 1	71	0.7	2.0	0.8
Group 2	9	1.3	6.0	2.5
Group 3	1	3.0	14.0	5.5
All	81	0.8	2.5	1.1
Male and Female	295	1.0	4.5	2.2

Only 2.4 per cent gave a forty-five minute reading above 15 mm.

If the rate is above 5 mm. or if the forty-five minute reading is above 15 mm., examine the patient for a cause of increased rate.

Menstruation seems to have little effect on the rate, low rates being secured in those (about 20) who were menstruating.

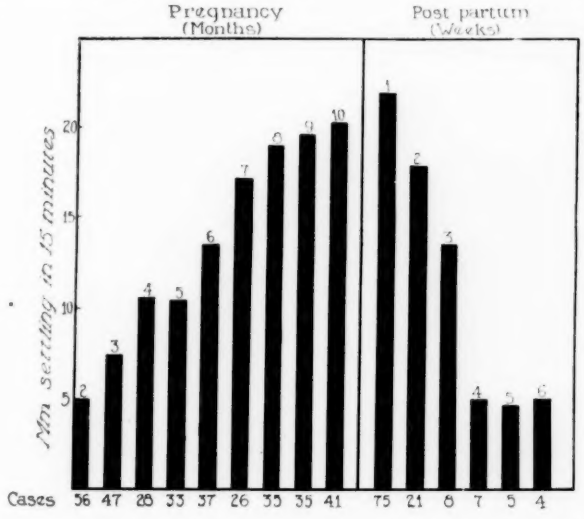

Fig. 3.—Sedimentation rates in prenatal and uncomplicated postpartum cases.

Pregnancy and Postpartum Findings.—These are shown in Fig. 3. We secured the same type of results as have been secured by others.

Sedimentation in Gynecologic Cases.—The results are shown in Table II; the rates were definitely increased in infective processes, abortion, and ma-

TABLE II

SEDIMENTATION IN GYNECOLOGIC CASES

NO. OF CASES	DIAGNOSIS*	NO. OF CASES HAVING NORMAL SEDIMENTATION	AVERAGE 15 MIN. READING MM.	AVERAGE 45 MIN. READING MM.	AVERAGE RATE MM.
138	Salpingitis	14	17	57	23.5
62	Abortion	6	13	51	20.0
8	Tubal pregnancy (ruptured)	1			12.0
39	Fibroid	8	7	26	8.5
14	Malignancy	3	21	60	27.0
4	Pelvic abscess	0	23	62	27.0
17	Ovarian cysts	16			5.5
48	Cervical erosions	37			3.5
40	Vaginitis (trichomonas vag.)	21			4.0
23	Infection of Skene's or Bartholin's glands	0			11.0
12	Endometrium hyperplasia	2			8.5
13	Sterility	11			3.0
6	Asthenia	6			2.0
12	Fröhlich's syndrome	8			4.5
1	Subinvolution	0			22.0
183	Miscellaneous	54			Varying

*Rates of cases complicated by other conditions than the diagnosis given, were not used in calculating the averages.

TABLE III

SEDIMENTATION IN MEDICAL AND SURGICAL CASES

NO. OF CASES	DIAGNOSIS*	NO. OF CASES HAVING NORMAL SEDIMENTATION	AVERAGE 15 MIN. READING MM.	AVERAGE 45 MIN. READING MM.	AVERAGE RATE MM.
26	Bronchopneumonia				32
12	(a)	0	8	43	18
14	(b)	0	33	89	38
7	Lobar pneumonia	0	27	83	36
12	Bronchitis				25
3	(a)	1	5	15	6
9	(b)	0	21	70	30
9	Acute infection of upper respiratory tract				20
5	(a)	4	4	14	10
4	(b)	0	22	87	34
2	Pleurisy	0	26	77	29
7	Acute rheumatic arthritis	0	42	96	48
13	Rheumatic heart disease				17
8	(a)	2	4	20	8
5	(b)	0	20	74	26
4	Subacute bacterial endocarditis	1	7	40	16
2	Arthritis deformans	0	37	97	41
8	Appendicitis, not acute (no leucocytosis)	4	3	13	5
3	Appendicitis and peritonitis	1	6	29	11
7	Otitis and mastoiditis	0	15	55	21
7	Sinusitis	0	29	69	34
2	Tonsillitis	0	38	92	41
12	Abscesses	0	20	69	29
7	Acute genitourinary inflammation	0	31	76	34
12	Local infections and inflammation	2	17	50	22
3	Burns of extremities	1	12	40	14
5	Ulcers of extremities	1	16	47	18
13	Peptic or duodenal ulcers	6	4	16	7
8	Carcinoma of stomach	1	12	45	16
12	Carcinoma, other cases	2	13	41	18
4	Tumors, not carcinoma	1	22	49	22
10	General arteriosclerosis				19
2	(a)	1	4	13	11
8	(b)	0	16	55	21
9	Arteriosclerosis with heart, brain or other complications	2	6	25	10
8	Cerebral hemorrhage or thrombosis	1	4	25	10
24	Hypertension and hypertensive cardiovascular renal disease				11
11	(a)	8	2	9	7
13	(b)	0	10	47	18
9	Syphilis				14
4	(a)	3	3	10	4
5	(b)	0	8	51	22
	Pernicious anemia				
3	(a) R.B.C. above 2.6 mil.		5	19	7
5	(b) R.B.C. below 2.6 mil.		11	48	19
7	Diabetes mellitus	1	7	31	13
3	Enteritis and colitis	0	16	77	31
2	Amebic dysentery	0	24	60	25
3	Meningitis	1	5	27	22
4	Tabes dorsalis	3	3	9	4
12	Postoperative results	0	26	80	33

TABLE III—CONT'D

NO. OF CASES	DIAGNOSIS*	NO. OF CASES HAVING NORMAL SEDIMENTATION	AVERAGE 15 MIN. READING MM.	AVERAGE 45 MIN. READING MM.	AVERAGE RATE MM.
19	Hernia	14	4	10	4
14	Fractures				20
5	(a)	0	4	20	8
9	(b)	0	17	59	26
9	Hypertrophy of prostate				15
3	(a)	3	2	6	2
6	(b)	0	12	55	22
6	Gonorrhea, not acute	3	3	13	5
10	Chronic cholecystitis				8
7	(a)	6	2	7	3
3	(b)	0	6	38	16
7	Kidney cases not nephritic	2	11	39	15
15	Tuberculosis				22
4	(a)	3	3	13	6
11	(b)	0	20	66	26
6	Lung cases, miscellaneous	2	5	27	11
11	Heart cases, noninfective	6	4	29	12
11	Thyroid cases	4	6	25	10
13	Miscellaneous nervous and mental cases	5	5	20	8
6	Miscellaneous eye cases	4	3	17	7
194	Miscellaneous cases	51			17
51	(a)	51			3
60	(b)	0			11
60	(c)	0			24
23	(d) Most had infective process	0			46

*Rates of cases complicated by other conditions than the diagnosis given, were not used in calculating the averages.

lignancy. The obstetric and gynecologic findings are fully discussed in another paper.[2]

Sedimentation in Medical and Surgical Cases.—Table III reports our findings. There is a slight or moderate increase in sedimentation rate in a large number of conditions. The highest rates were shown in pneumonias, acute inflammations and infections, acute rheumatic fever, infectious arthritis, fractures, and as postoperative effects.

DISCUSSION

Practically all patients admitted to the local County Hospital for a six-month period were tested for their blood sedimentation rates. The proportion of surgical cases was not large. There were few tuberculous or syphilitic cases.

The gynecologic and obstetric cases were increased by a considerable number from the private practice of one of us (A. M.).

A large number of cases had to be put into the miscellaneous group because there was more than one disease condition. Only cases in which complications were not found were grouped under a particular diagnosis in making the tables. However, in the class of patients that are admitted to a county hospital, it is probable that focal infection (teeth, tonsils, sinuses, etc.) was not infrequently present when not recorded in the clinical diagnoses; hence,

some of the rates may be too high for *uncomplicated* cases of the particular disease condition in the strictest interpretation of that term.

Where there were a number of cases under one diagnosis, we have separated them into two groups, the slower and the faster settling bloods, so that the frequency of each type of rate may be judged. Also we have indicated the number of cases that had a normal rate.

A paper analyzing the clinical data in more detail will appear later.

CONCLUSIONS

1. A simple, rapid method using oxalated venous blood is described.

2. This method has definite advantages over previously described methods, and gives a better idea of the actual sedimentation rate.

3. The results are secured in forty-five minutes.

4. Tables of findings in a large number of normal and clinical cases are given.

5. The test is so simple that it could be done to advantage routinely, and repeated at frequent intervals.

Its chief value is not in differential diagnosis but in following the course of the individual case and in giving a clue to unsuspected infection, inflammation, or malignancy in persons in whom a normal rate had been expected.

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THE REDUCTION OF PHOSPHOMOLYBDIC ACID BY MONOMETHYL-PARAMIDOPHENOL AND ITS USE IN THE QUANTITATIVE DETERMINATION OF PHOSPHORUS IN BLOOD*

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PHOSPHOMOLYBDIC acid is reduced by certain reagents, under proper conditions, to a blue oxide. A number of methods using varying reducing agents, have been devised for the quantitative determination of small amounts of phosphorus. Taylor and Miller[1] used phenyl hydrazine as a reducing agent. Bell and Doisy[2] used hydroquinone. The method of Bell and Doisy was further modified by Briggs,[3] and by Benedict and Theis.[4] Fiske and Subbarow[5] introduced a new reducing agent, aminonaphtholsulphonic acid. Kuttner and Cohen[6] used stannous chloride.

To obtain quantitative results, the concentration and proper proportion of the reagents must be strictly adhered to; especially does this apply to the proper acidity. This is particularly the case with stannous chloride where the range of acidity is very limited. Failure to observe the proper conditions will affect a reduction of molybdic as well as phosphomolybdic acid. The reducing agents mentioned above are very unstable and deteriorate rapidly.

In searching for a more stable reducing agent a number of phenol derivatives have been tested and one of these, monomethylparamidophenol, was found to be very efficacious for the reduction of phosphomolybdic acid. This reagent is very stable in the presence of sodium sulphite; it produces a good blue color in the cold; and when properly used will reduce phosphomolybdic acid only.

Procedures are here described for the quantitative determination of inorganic phosphate, total acid-soluble phosphorus, and lipoidal phosphorus in small amounts of blood.

DETERMINATION OF INORGANIC PHOSPHATE IN BLOOD SERUM

Principle.—The proteins are removed with trichloracetic acid and an aliquot portion is treated with ammonium molybdate and monomethylparamidophenol-sodium sulphite. The blue color thus produced is matched in the colorimeter against a standard phosphate solution.

Procedure.—To 2 c.c. of serum in a test tube are added 4 c.c. of distilled water and 4 c.c. of 10 per cent trichloracetic acid. The contents of the tube are well mixed and after standing for ten minutes are filtered through an ashless filter paper. To 5 c.c. of the filtrate are added 3 c.c. of water, 1 c.c. of ammonium molybdate, and 1 c.c. of monomethylparamidophenol reagent.

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The contents are mixed by inverting the tube. After standing at room temperature for five minutes it is compared in the colorimeter against a standard phosphate solution.

The standard is prepared as follows: 5 c.c. of the standard phosphate solution are placed in a test tube. To this are added 3 c.c. of 10 per cent trichloracetic acid solution and 1 c.c. each of ammonium molybdate and monomethylparamidophenol. The latter two reagents are added to the standard and to the unknown at the same time. The standard is placed in the colorimeter at 15 or 20 mm.

Calculation of Results:

$$\frac{S}{R} \times 4 = \text{mg. phosphorus in 100 c.c. of serum.}$$

S = reading of standard.
R = reading of unknown.

PREPARATION OF REAGENTS

1. Trichloracetic Acid.—A 10 per cent solution in water. This reagent must be of the highest degree purity. It should be distilled. Most samples of trichloracetic acid contain small amounts of phosphate. Martland and Robison[7] also found an organic impurity which reduces molybdic acid. This impurity they completely removed by distillation.

2. Molybdate Solution.—Thirty gm. of ammonium molybdate are dissolved in 500 c.c. of water.

3. Monomethylparamidophenol Reagent.—Fifty gm. of sodium sulphite are dissolved in 500 c.c. of water. To this are added 5 gm. of monomethylparamidophenol sulphate and shaken until completely dissolved. This reagent will keep for at least six months.

4. Standard Phosphate Solution.—0.3509 gm. of pure KH_2PO_4 is dissolved in water and made up to 1 liter in a volumetric flask and preserved with chloroform. This is the stock solution. The standard for use should contain 0.04 mg. of phosphorus in 5 c.c. of solution. It is prepared by diluting the stock solution 1 to 10 and preserving with chloroform.

DISCUSSION

The concentration and proportion of the various reagents used in the test have been adjusted to produce the maximum intensity and stability of color at room temperature. The color is specific for phosphomolybdic acid; it develops very rapidly and is stable for many hours. The color is quite independent of the degree of acidity within wide limits. This is very important since it is not possible to reproduce exactly the degree of acidity in the standard and the unknown due to the trichloracetic acid used in the removal of protein. A certain amount of acid is necessary to form phosphomolybdic acid which is subsequently reduced to the blue oxide. In the absence of acid no blue color develops. If less than the minimum amount of acid is present

the blue color does not develop fully and is of a somewhat different hue. A great excess of acid retards the development of the color and diminishes its intensity.

The effect of the degree of acidity upon the development of the color is shown diagrammatically in Fig. 1 where colorimetric readings are plotted against acidity. The range of acidity lies between 0.3 and 1.8 normal as is shown by the straight line obtained within this range. No sulphuric acid is used in this test; the required acidity being maintained by the trichloracetic acid used in the precipitation of the protein.

Table I shows the recovery of added phosphate. The results are recorded in milligrams of phosphorus per 100 c.c. of serum.

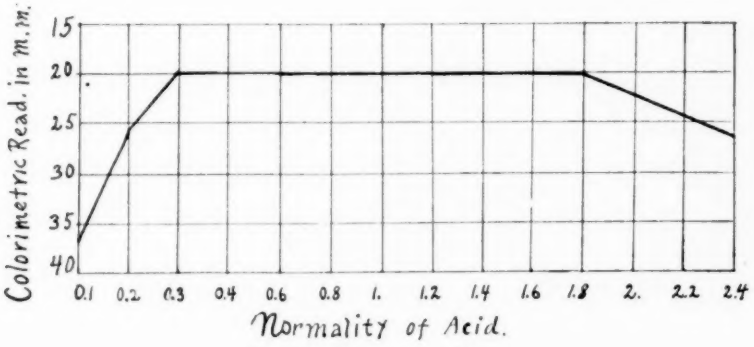

Fig. 1.—Range of acidity.

TABLE I

RECOVERY OF ADDED PHOSPHATE

TUBE	P IN SERUM	ADDED P MG. PER 100 C.C.	TOTAL P	RECOVERED P
1	4.1	none	4.1	4.1
2	4.1	2.5	6.6	6.4
3	4.1	5.0	9.1	8.9
4	4.1	10.0	14.1	13.9
5	4.1	15.0	19.1	18.8

DETERMINATION OF TOTAL ACID-SOLUBLE PHOSPHORUS IN SERUM

Principle.—Organic matter is destroyed in the trichloracetic acid filtrate with sulphuric acid and hydrogen peroxide and the phosphorus determined as in the preceding method.

Procedure.—Two c.c. of the trichloracetic acid filtrate (prepared as previously described) are placed in a 20 c.c. volumetric Pyrex flask. To this is added 0.5 c.c. of concentrated sulphuric acid and the flask is heated on the sand-bath until SO_3 fumes fill the mouth of the flask. The mouth of the flask is then covered with a small beaker and heating is continued for five to ten minutes. The flask is then allowed to cool and 5 drops of 30 per cent hydrogen peroxide (Merck "Special") is added and heating is continued for five minutes after SO_3 fumes are given off. Usually the solution will be perfectly

clear and colorless. Should there remain a brownish color, one or two more drops of hydrogen peroxide should be added and the heating repeated until a clear solution is obtained.

Preparation of Standard.—In a similar 20 c.c. volumetric flask are placed 0.5 c.c. of concentrated sulphuric acid and 5 drops of hydrogen peroxide and boiled on the sand-bath for five minutes in the same way as the trichloracetic acid filtrate. When cool, 10 c.c. of the standard phosphate solution are added (the standard is the same as used for the inorganic phosphate). To both the standard and the unknown are added about 10 c.c. of water, 2 c.c. of ammonium molybdate solution and 2 c.c. of monomethylparamidophenol reagent. Water is then added to the 20 c.c. mark. The solutions are mixed well and after standing for five minutes are compared in the colorimeter, setting the standard at 15 or 20 millimeters.

Calculation of Results:

$\frac{S}{R} \times 20 =$ mg. phosphorus in 100 c.c. of serum. (Merck "Special" hydrogen peroxide must be used as other brands contain traces of phosphates.)

DETERMINATION OF LIPOIDAL PHOSPHORUS IN BLOOD

Principle.—The lipoids are extracted with an alcohol-ether mixture and the extract is oxidized with sulphuric acid and hydrogen peroxide. The phosphorus is then determined as in the previous methods.

Procedure.—Twenty c.c. of a mixture of 3 parts alcohol and 1 part ether are introduced in a 25 c.c. volumetric flask. While rotating the flask 1 c.c. of well mixed oxalated blood is added slowly drop by drop, to prevent the formation of large clumps. The flask is then immersed in a large beaker of hot water with frequent and vigorous shaking until the liquid in the flask just begins to boil. It is then cooled to room temperature by immersing in cold water. Alcohol-ether mixture is added to the 25 c.c. mark and after shaking is filtered through an ashless fat-free filter paper, the funnel being covered with a watch glass to prevent evaporation.

Ten c.c. of the filtrate are transferred to a 20 c.c. volumetric Pyrex flask. To this is added 0.5 c.c. of concentrated sulphuric acid. The flask is heated on the sand-bath until all the solvent is boiled off and SO_3 fumes begin to come off. The flask is then covered with a small beaker and heating continued for five to ten minutes longer. The flask is then allowed to cool and 5 drops of 30 per cent hydrogen peroxide are added and the heating continued for five minutes after SO_3 fumes are given off. When cool, 10 c.c. of water are added.

Preparation of Standard.—In a similar 20 c.c. volumetric flask are placed 10 c.c. of the alcohol-ether mixture, 0.5 c.c. of concentrated sulphuric acid, and 5 drops of 30 per cent hydrogen peroxide. The mixture is heated for five minutes after SO_3 fumes are given off. When cool, 10 c.c. of the standard phosphate solution are added (the same standard as in the other two tests is used).

To both the standard and the unknown are now added 2 c.c. of ammonium molybdate and 2 c.c. of monomethylparamidophenol reagent, and diluted with water to the 20 c.c. mark. The contents of the flasks are well mixed and after standing for five minutes at room temperature are compared in the colorimeter setting the standard at 15 or 20 millimeters.

Calculation of Results:

$$\frac{S}{R} \times 10 = \text{mg. phosphorus in 100 c.c. of blood.}$$

DISCUSSION

Hydrogen peroxide is chosen as an oxidizing agent in preference to nitric acid as it is more effective. It is very difficult to remove the last traces of nitric acid, and when not completely removed it interferes with the development of the blue color and produces cloudy solutions. One has to guard, however, against the use of any other brand of hydrogen peroxide than specified, as other brands contain traces of phosphates.

The use of flasks and the sand-bath is a great advantage in the evaporation of the alcohol-ether mixture as boiling proceeds very smoothly and no loss of material is possible, as is often the case when evaporation is done in the water-bath where violent bumping often occurs thus causing a loss of material.

The amount of sulphuric acid lies well within the proper range.

SUMMARY

Monomethylparamidophenol was found to be a good reducing agent for phosphomolybdic acid. With the use of this reagent methods are described for the quantitative determination of inorganic phosphate, total acid-soluble phosphorus, and lipoidal phosphorus in small amounts of blood.

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THE DETERMINATION OF BLOOD PROTEINS BY A DIRECT MICRO KJELDAHL METHOD*

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VARIOUS methods for the determination of the protein content of blood are available, each of which is adapted to a different type of laboratory equipment. It is believed that the one described below will be found useful especially in laboratories where the analysis is carried out only occasionally and where micro Kjeldahl methods form the standard procedure for nitrogen determinations. Since in this method some of the protein values are calculated from differences between figures obtained in nitrogen analyses, the procedure is not as satisfactory as are those where precipitated proteins are determined directly.

Hawk[1] has described a micro Kjeldahl method for this purpose combining the technic of Howe,[2] Wu,[3] and Koch and McMeekin.[4] He separated serum albumin from serum globulin with sodium sulphate, and precipitated fibrinogen from plasma by the action of calcium chloride. A series of micro Kjeldahl analyses were then carried out using hydrogen peroxide and sulphuric acid containing a small amount of copper sulphate as the oxidizing reagents. The nitrogen content was determined by direct nesslerization. Protein values† were calculated in part from differences between the values obtained.

When this method was applied precipitation of Nessler's reagent was not infrequently encountered, so that it was often necessary to repeat the whole procedure before satisfactory results were obtained. For four years there has been in use in this laboratory a direct nesslerization method for the determination of urea in blood filtrates which was recommended to us by Dr. James B. Sumner. In this method Rochelle salt is used to prevent the precipitation of Nessler's reagent. This technic has proved very satisfactory, and it seemed worth while to try the effect of Rochelle salt upon the final determination in solutions prepared as described by Hawk. Accordingly this was done, and the results were found to be very satisfactory. The only other modification made was to reduce the amount of material taken for analysis. This change lessened the amount of hydrogen peroxide needed and so decreased the chance of losing solution through spattering when the reagent was added to the hot digestion mixture. The details of the procedure are given below. They are almost identical with those described on pages 399 and 400 of Hawk and Bergeim's book[1] except for the modifications just noted.

Reagents.—(1) 0.9 per cent sodium chloride; (2) 2.5 per cent calcium chloride; (3) concentrated sodium sulphate, prepared as follows: dissolve 22.2 gm. of the anhydrous salt in 75 to 90 c.c. of hot distilled water, pour into a

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†See Myers, V. C.: Chemical Changes in the Blood in Disease. I. Nonprotein and Urea Nitrogen, J. Lab. & Clin. Med. 5: 418-424, 1920.

flask graduated at 100 c.c. and place in an incubator at 37° C.; place in incubator some distilled water; when the temperature of the incubator is reached by both liquids, dilute the sodium sulphate to the mark with the distilled water; the solution is supersaturated at room temperature, and must be kept in the incubator. (4) Digestion mixture: to 10 c.c. of 5 per cent copper sulphate solution add 100 c.c. of concentrated sulphuric acid as free from nitrogen as possible; pour with stirring into 100 c.c. of distilled water. (5) caprylic alcohol. (6) Strong (30 per cent) hydrogen peroxide: this reagent is usually preserved by the addition of small amounts of compounds containing nitrogen, and a correction must be made for this nitrogen. The correction is determined in the following manner: to 1 c.c. of boiling digestion mixture add 30 drops of the hydrogen peroxide; after digestion is complete, cool, and nesslerize as described below; the nitrogen content divided by 30 will give the nitrogen added in each drop of the peroxide. By special arrangement with the manufacturers it is sometimes possible to obtain peroxide which does not contain preservative; if this is kept in colored bottles on ice it will retain sufficient strength for at least three years to meet the needs of the method; the use of such a reagent free from nitrogen greatly simplifies the calculation. (7) Rochelle salt: a 10 per cent solution. (8) Nitrogen standard: a convenient dilute working nitrogen standard contains 1 mg. of nitrogen in the form of ammonium sulphate in 20 c.c. of solution. Prepare a strong solution by weighing 11.79 gm. of pure ammonium sulphate and dissolving in 500 c.c. of distilled water. Dilute 10 c.c. of this to 1000 c.c. in a standard volumetric flask to make the working standard. (9) Nessler's solution: prepare according to the directions of Folin and Wu[5]; 150 gm. of potassium iodide and 110 gm. of iodine are placed in a 500 c.c. Florence flask; 100 c.c. of water and an excess (140 to 150 gm.) of metallic mercury are added; the flask is then shaken continuously for seven to fifteen minutes until the color of iodine has disappeared and is replaced by the greenish color of the double iodide. The solution, which becomes quite hot, must be cooled under the tap when the color begins to get pale and before the red has wholly disappeared. Then decant, wash the mercury and flask with a large volume of water, and make the supernatant fluid and washings up to two liters. The final Nessler's solution is prepared from this double iodide and from 10 per cent sodium hydroxide which should be standardized with an accuracy of at least 5 per cent and which must be free from carbonate. To ensure the latter, prepare a strong solution of sodium hydroxide containing 55 gm. in each 100 c.c. and allow to stand for several days or weeks until the sodium carbonate has precipitated and settled; then decant and dilute to a strength of 10 per cent just before using. To prepare the final Nessler's solution mix 3500 c.c. of this 10 per cent sodium hydroxide, 750 c.c. of the double iodide solution described above, and 750 c.c. of distilled water. The alkalinity of this solution is important; 20 c.c. of normal hydrochloric acid should require between 11 and 11.5 c.c. of the finished Nessler's solution to give an endpoint with phenolphthalein. The solution is clear when first prepared; if a precipitate forms, the supernatant fluid may safely be decanted and used.

Preparation of Solutions.—For total protein dilute one c.c. of plasma or serum accurately to 50 c.c. with 0.9 per cent sodium chloride. Take 0.5 c.c.,

measured with an Ostwald pipette, and proceed with the digestion as described below. To precipitate fibrin add exactly 48 c.c. of 0.9 per cent sodium chloride and 1 c.c. of 2.5 per cent calcium chloride to 1 c.c. of plasma in a 50 c.c. glass cylinder. Let stand until the clot has formed (twenty minutes or more), break up or remove the clot with a glass rod, and filter. Take 0.5 c.c. and digest. Determine albumin after removing globulin in the following manner. Treat 1 c.c. of serum or plasma with 30 c.c. of the 22.2 per cent sodium sulphate solution. Stopper, and let stand in the incubator for three to four hours. Filter in the incubator, using a good grade of filter paper, and protecting against loss by evaporation. The funnel should be placed directly in the mouth of a test tube or flask, and the top of the funnel should be covered with a watch glass. If the filtrate is not perfectly clear return to the filter paper. Take 0.5 c.c. of the filtrate, which contains albumin but not globulin, and proceed with the digestion.

Digestion.—Measure 0.5 c.c. quantities of the different solutions and filtrates described in the preceding paragraph into Pyrex test tubes graduated at 35 and 50 c.c. Add a few chips of broken porcelain, a small drop of caprylic alcohol and 1 c.c. of the acid digestion mixture. Boil over a micro burner until the water has been driven off and white fumes appear. Insert a small funnel in the mouth of the tube and reduce the flame. Continue heating until marked charring is produced and dense white fumes fill the tube. Allow to cool for thirty seconds and then add the peroxide carefully a drop at a time. If peroxide free from nitrogen is not available the number of drops of the reagent used should be counted. Boil again for one minute, and cool thoroughly. Add a little water, 1 c.c. of 10 per cent Rochelle salt and make to a volume of 35 c.c. with distilled water. For the standard take 3 c.c. of the dilute ammonia solution equivalent to 0.15 mg. of nitrogen (if the protein content is very low it may be advisable to prepare a second standard containing 2 c.c. of the dilute solution which is equivalent to 0.10 mg. of nitrogen) and 1 c.c. of the oxidizing solution, 1 c.c. of 10 per cent Rochelle salt, and make to a volume of 33 c.c. Add 15 c.c. of Nessler's solution to the standard and unknown, mix thoroughly and compare in a colorimeter. The standard is most conveniently set at 20 mm.

Calculation.—The protein values are calculated from the nitrogen present by multiplying by the factor 6.25. The calculation follows.

$$\frac{S}{U} \times 0.15^{*} - nE^{\dagger} = \text{mg. nitrogen found.}$$

$$\text{Mg. nitrogen} \times \frac{100}{V} = \text{mg. nitrogen per 100 c.c. serum, plasma, etc.}$$

$$\text{Mg. nitrogen per 100 c.c.} - \text{NPN} = \text{mg. protein nitrogen per 100 c.c. serum, etc.}$$

$$\text{Mg. protein nitrogen} \times \frac{6.25}{1000} = \text{gm. protein per 100 c.c. serum, etc.}$$

S = reading of standard; U = reading of unknown; n = number of drops of peroxide used; E = nitrogen content of each drop; V = actual volume of plasma, serum, or, for albumin and fibrinogen, the equivalent of the amount of serum and plasma taken for each determination; and NPN = the nonprotein nitrogen present expressed as mg. per 100 c.c. For total protein in serum

*0.15 represents the usual nitrogen standard, i. e., 3 c.c. of the dilute standard solution. If 2 c.c. are used because readings are otherwise too low the figure is 0.10.

†If peroxide free from nitrogen is used the term "nE" is omitted.

and plasma, and for the determination upon filtrate from which fibrinogen has been removed V = 0.01. For albumin V = 0.0165 (i.e., 0.5 x 1/31): Total protein in serum or plasma, and albumin (in the filtrate from precipitation with sodium sulphate) are determined directly by the formula given. Fibrinogen = total plasma protein – protein in fibrinogen filtrate after treatment with calcium chloride. Globulin = total serum – serum albumin; or globulin = total plasma protein – plasma albumin – fibrinogen.

SUMMARY

A micro Kjeldahl method for determining proteins in blood is described depending upon the principle of direct nesslerization after oxidation. Rochelle salt is used to prevent precipitation of the Nessler's reagent.

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A NOTE ON THE GUNTHER-GREENBERG METHOD*

For Determining Inorganic Phosphorus on the Filtrate From Calcium Analysis

By Ruth Purrier Bolton, Los Angeles, Calif.

IN COMMON with other laboratory workers, we appreciate any practical means of reducing the volume of blood required for determining a number of blood constituents. Gunther and Greenberg[1] have published a method by means of which inorganic phosphorus may be determined on the supernatant solution from the calcium precipitation of the Modified Kramer Tisdall[2] method. They oxidize the oxalate remaining in solution with potassium permanganate and subsequently determine the phosphorus by the Fiske-Subbarow method. We have found the modification equally practical with the hydroquinone bisulphite reagent of the Benedict-Theis[3] modification of the Briggs[4] method.

EXPERIMENTAL

With the following procedure we obtain results which check satisfactorily with determinations made on whole serum: Into a 15 c.c. centrifuge tube accurately pipette 2 c.c. of distilled water, 2 c.c. of serum, and 1 c.c. of 4 per cent ammonium oxalate. Twirl the tube to mix, and allow to stand for at least one hour. Centrifuge for ten minutes, pour the supernatant fluid into

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another tube and reserve for the phosphorus determination. Wash and titrate the calcium oxalate as usual. Transfer 4 c.c. of the supernatant solution to a 100 c.c. Erlenmeyer flask. Dilute with 3 c.c. of distilled water and add slowly 3 c.c. of 20 per cent trichloracetic acid. Shake well and allow to stand for ten minutes. Filter through ashless, acid-washed filter paper (Whatman No. 42). Transfer 5 c.c. of the filtrate to a 25 c.c. test tube, add 1 c.c. of distilled water, 1 c.c. of the molybdic acid reagent (which has been diluted 1:1 with concentrated sulphuric acid), and 2 c.c. of N/10 potassium permanganate. Allow to stand until the color disappears and then add 1 c.c. of the hydroquinone bisulphite reagent. Mix and stopper loosely. Simultaneously prepare the standard as follows: In a second 25 c.c. test tube place 5 c.c. of the standard solution of potassium biphosphate (containing 0.025 mg. of phosphorus in each 5 c.c.), and add 3 c.c. of distilled water, 1 c.c. of the molybdic acid reagent and 1 c.c. of the hydroquinone bisulphite reagent. Mix and stopper loosely. Heat both tubes in a boiling water-bath for ten minutes. Cool to room temperature and read in the colorimeter.

CALCULATION

The 4 c.c. portion of supernatant fluid represents 1.6 c.c. of serum and the 5 c.c. of filtrate represents 0.8 c.c. of serum. If the standard, which contains 0.025 mg. of phosphorus, is set at 20 mm. for the colorimeter comparison, the calculation is as follows:

$$\frac{20 \text{ mm.}}{\times} \times \frac{10 \text{ c.c.}}{10 \text{ c.c.}} \times 0.025 \text{ mg.} \times \frac{100 \text{ c.c.}}{0.8 \text{ c.c.}} = \text{mg. of phosphorus per 100 c.c. of serum}$$

CONCLUSION

The Gunther-Greenberg Method for determining inorganic phosphorus on the filtrate from the calcium analysis has been satisfactorily applied, using the Benedict-Theis Method for the inorganic phosphorus determination.

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A GRAPHIC METHOD FOR MEASURING THE CLOTTING TIME OF BLOOD*

By R. H. K. Foster, Chicago, Ill.

MANY types of instruments have been devised to measure the clotting time of blood and the results are comparable only when measured by the same method. The vast majority of the methods described in the literature employ blood obtained by cutaneous puncture. The present method employs venepuncture. In any method the essential plan is to preserve the constancy of conditions. Addis[1] gives the following conditions for an accurate determination of the coagulation time:

1. The blood must be obtained under the same conditions in each experiment;

2. The blood must come in contact with the same kind and amount of foreign bodies in each observation;

3. All estimations must be made at the same temperature;

4. The end-point must be clear and definite and must always indicate the same degree of coagulation.

The apparatus herein described fulfills these conditions, the one point where error is likely is in actually taking the sample of blood. As is well known tissue juices play an important part in the coagulation as is evidenced by the fact that samples taken cutaneously give less than half the clotting time as do samples taken directly from a vein.

The present method gives a graphic record of the rate of the clotting process. It is somewhat similar to the graphic method of Mendenhall and Cannon[2] though it differs in several details.

APPARATUS

The principle upon which the method depends is that as the clotting process proceeds the viscosity of the blood increases, and it is this increase in viscosity which is measured, though not in actual units. The present method was gradually evolved from an idea suggested by the tensiometer.

Briefly, the method consists in placing an accurately measured volume of blood (0.30 c.c.) between two discs of unequal diameter, the upper being the smaller, and at regular intervals measuring the displacement of the upper movable disc when actuated by a given constant weight.

Fig. 1 illustrates the apparatus diagrammatically.

The lower stationary disc is a ¾ in. glass cover slip mounted with balsam on a brass base *G*. A stream of water at constant temperature flows through

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base *G* as indicated by the arrows and comes in direct contact with the under side of the cover slip. Since the film of blood is thin (about 1 mm.) the temperature throughout is substantially constant.

The upper disc is of aluminum and is $\frac{3}{8}$ in. in diameter and $\frac{1}{32}$ in. in thickness. It is fastened to a rod which is suspended from one end of an aluminum beam. The opposite end of this beam is attached by means of a thread to a somewhat longer lever. By placing the respective fulera as indicated a great amplification of the displacement of the disc is obtained by the writing point.

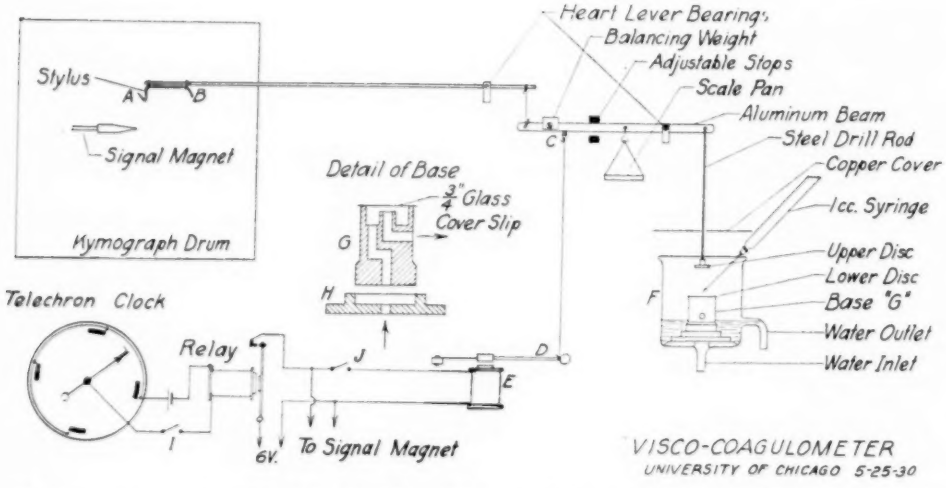

Fig. 1.—Diagram of the apparatus used in making a graphic record of the clotting time of blood. See text for explanation.

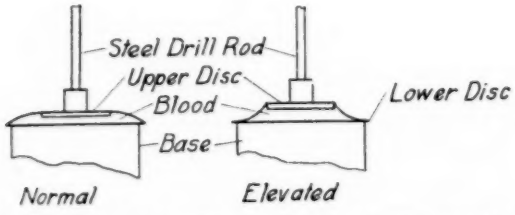

Fig. 2.—Diagram illustrating the position of the discs in the normal and raised conditions. The contour of the surface of the blood is indicated and is convex in the normal and concave in the elevated positions. The normal position was of twelve seconds' duration and the elevated three seconds' duration.

The writing point (*A*, *B*) was designed to minimize friction and equalize pressure on the drum. A small glass tube, drawn out to a fine capillary bore, is bent so the point of the stylus *A* rests vertically against the drum. The other end *B* is horizontal and away from the drum so that its weight tends to hold the writing point against the drum. The stylus is supported by two fine wires in which it can revolve freely, though without end-play. The writing point itself (*A*) was firepolished an instant to give a fine ball point. With this type of stylus the friction becomes practically constant which is of course necessary to obtain constant end-points. There is considerable leeway in plac-

ing the drum against the writing point and variations of as much as ⅛ in. cause but a slight change in the resistance offered by the entire lever.

The system is accurately balanced by a movable weight on the aluminum beam and then a definite weight is placed in the scale pan. Experience has indicated 0.3 gm. to be the most appropriate counterbalancing weight. This weight pulls down on the aluminum beam thus raising the upper disc, and moving the writing point over the smoked drum which rotates at the slowest speed. Two adjustable stops limit the excursion of the levers. A flexible rod *C* (a wooden swabstick being convenient) supports the lever in its resting position and when this is released the levers move to their maximum position. A string is attached to this flexible rod *C* and to the striker *D* of a doorbell or interrupter *E*. The interrupter was rewired to give an uninterrupted magnetic field. The aluminum beam is released by sending a current through the magnet *E* at regular intervals. An electric timer is used to regulate the impulses actuating the striker of the magnet. The timer consists of a Telechron electric clock on which platinum contacts have been placed at the fifteen second intervals. The contacts cover a space of three seconds. A platinum contact wire is soldered to the second hand of the clock. Thus electric impulses are obtained every fifteen seconds for a period of three seconds each. To insure safety and more satisfactory operation a relay is placed between the clock and the magnet.

The base *G* to which the lower disc is fastened rests in a depression in a brass plate *H* which is secured by beeswax in a specially designed Pyrex beaker *F*. The beaker is clamped to the movable carriage of a rack and pinion stand. The beaker can thus be raised or lowered over a distance of 3 or 4 inches vertically.

In operating, the sample of blood is put on the lower disc in the lowered position (as indicated in Fig. 1) and the beaker is then quickly raised so the two discs are 1 mm. apart. This distance is predetermined and a collar on the rack and pinion stand is set so as to stop the disc in the required position. The blood assumes a position shown in Fig. 2, normal. It should be noted that the blood never creeps over the top of the upper aluminum disc or over the edge of the lower glass disc.

The copper cover indicated in the sketch is simply to prevent air currents from causing excessive evaporation or changes in temperature of the sample. Because of the water in the bottom of the beaker the humidity is fairly high thus minimizing evaporation of the sample. The temperature of the surrounding air is usually but a few degrees below that of the circulating water. As 37° was the temperature employed, the air temperature in the beaker varied from about 30° to 34° depending largely on room temperature.

EXPERIMENTAL PROCEDURE

The greater part of the work for which this apparatus was designed was done on dogs. Samples were drawn from the saphenous vein with a 1 c.c. Luer type tuberculin syringe. A 1½ inch No. 20 needle was used. Extra needles and syringes were kept previous to use at a constant temperature of 37° C. in a special compartment built in the side of the water-bath. Syringes

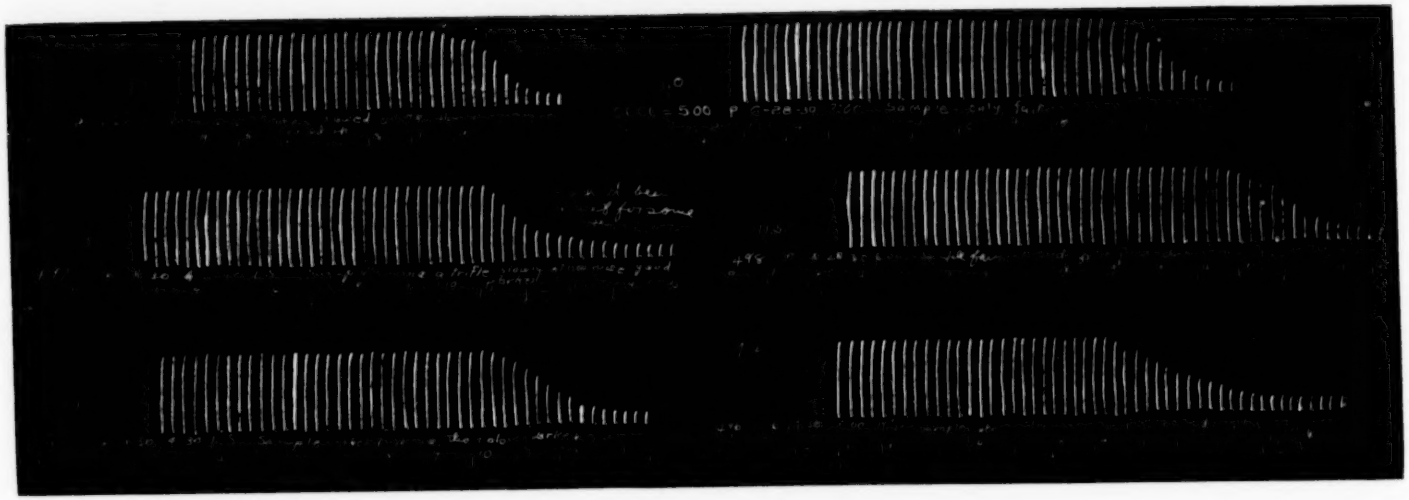

Fig. 3.—Tracings of tests 495 to 500, Exp. 53.

Lower line shows time intervals of fifteen seconds.

Upper line shows the three minute latent or rest period followed by the excursions of the writing point at fifteen second intervals. These vertical excursions are of three seconds' duration. Excursions of writing point retouched with white ink.

The end-point is taken as the last maximum excursion or in some cases the succeeding one when the drop has been very slight and when it seemed apparent that had the test been taken a few seconds sooner a maximal excursion would have been obtained.

In the upper left-hand corner of each test is indicated the end-point. Below the tracing of the clotting blood is given the test number, dog's initial, date, time of day, source of blood (right or left saphenous vein) and notes on the reliability of the venepuncture as noted at time of making the puncture. Changes in color of the blood are also noted. (See also Fig. 4.)

Variations in the base line were due to slight play in the adjustable stops.

were moistened with paraffin oil and the needles kept well sharpened with an Arkansas stone. The leg was carefully shaved over the vein. The skin was washed before each series of tests and any blood remaining after a test wiped away.

Immediately before making the puncture the kymograph was started, the constant-temperature water started circulating, and a rubber ligature put on the dog's leg above the knee. As soon as the vein was somewhat distended the needle was thrust quickly into the vein toward the current and the sample drawn into the syringe in minimum time. An assistant pressed a key or stop-watch at the instant blood appeared in the syringe and as soon as the needle was withdrawn, released the ligature and held the leg, clamping his fingers firmly on the vein to prevent external or subcutaneous hemorrhage. In measuring the sample onto the lower disc the needle was held in the position shown

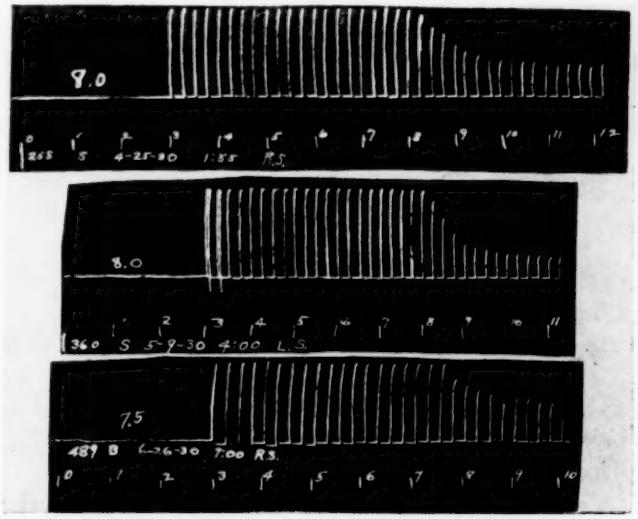

Fig. 4.—These tracings show records of tests from three different experiments and were chosen to show the variations in height of the excursions after the clotting process became substantially completed. Compare also with Fig. 4. The height is seen to be independent of the rapidity of the clotting process and is probably a measure of the amount of fibrin in the clot. In general the shorter the clotting time the steeper becomes the clotting curve as shown by comparing the above examples with those of Fig. 3.
Retouched with white ink.

in Fig. 1. Volume was measured to the nearest 0.01 c.c. using 0.30 c.c. The lower disc was immediately raised into position. This entire process from taking the sample usually consumed about twenty seconds. The magnetic release was started at the end of the third minute. This three minute rest period was chosen more or less arbitrarily, although a longer or shorter latent period could be used without affecting the accuracy although the absolute time would change somewhat but would be the same under identical conditions.

Without a doubt the taking of the sample is the most important part of the entire test. To minimize the error there involved, 1 c.c. or a trifle more was drawn into the syringe. The last 0.2 or 0.3 c.c. drawn from the vein were discarded and then 0.30 c.c. very accurately measured onto the lower disc. There then remained in the syringe 0.4 to 0.7 c.c. This represented the first

blood drawn from the vein and it is this first blood which is most contaminated with tissue juices and should be discarded. The advantage of using this type of syringe is twofold: being long and narrow there is less mixing of the blood than would occur in a shorter wider tube; the volume can be read with at least 5 times the accuracy of an ordinary 1 c.c. syringe.

The normal position of the sample of blood is shown in Fig. 2. When the rod *C* is drawn down the levers are released and the upper disc elevated as indicated. The blood flows in from the sides, making the surface concave instead of convex. As the viscosity increases, this flow becomes less and less until there is barely a perceptible motion of the upper disc. The displacement at first is about ¾ mm. and as the excursion of the writing point is 2 cm. the amplification is over 25 times.

When a certain point is reached in the clotting process the counter-balancing weight of 0.3 gm. becomes insufficient to overcome the resistance of the clotting blood and the excursion is submaximal gradually becoming less and less until a minimum level is obtained. The "end-point" is arbitrarily taken as the time to the last maximum excursion of the writing point. Just before the end-point is reached the levers move slower and slower and this change is seen on the tracing. After the end-point the excursions become less and less and a regular and uniform curve can be drawn through their apices. When clotting is complete this curve is horizontal. Thus we have a "clotting curve" the first part of which of course cannot be shown on the tracing, although the actual end of clotting is accurately recorded.

A further feature of the record is that we have an indication of the amount of fibrin in the clot. It is to be noted that in the sample tracings shown in Fig. 3 and Fig. 4 the excursions after completion of the clotting process vary in height with different animals. This is due to the varying strengths and elasticities of the clotted samples. Perhaps it is assuming too much to say that the amount of fibrin is proportional to the strength of the clot since the character of the fibrin shreds may vary and the same amount of fibrin produce a more tenacious clot at one time than another. It is believed, however, that the strength of the clot as herein indicated is a rough measure of the amount of fibrin in the blood. No work has been done to determine quantitatively the amount of fibrin as compared to the height of the final excursions of the writing point.

Samples which have a shorter clotting time, especially when there has been contamination with tissue juices, have a steeper clotting curve and usually in such samples a stronger clot is obtained.

The accuracy of the method is limited to fifteen seconds but the end-points are sharp and definite. Since coagulation is a continuous process there is no such thing as a true clotting time. The "end-point" is purely relative and is simply an arbitrarily chosen point on the clotting curve. And there is little to be gained from trying to reduce the accuracy to a shorter interval. Other factors such as difficulty in taking samples and changes in the blood itself far overshadow any attempts to employ a "precision" instrument. Although the test interval in this coagulometer could be reduced to ten or five seconds there is nothing to be gained thereby.

In dogs the normal clotting time by this method varies from about eight minutes to ten or eleven minutes. Rabbits have normals around ten and in one case fifteen minutes. The normals for dogs seem to rise gradually as they are kept penned up in kennels over a period of months. This may be due to insufficiently balanced diet with changes in the calcium and especially is it likely due to the lack of exercise affecting the general clotting mechanism.

A few typical series of tests are shown in Tables I and II. It must be remembered that in all these tests the quality of the sample may be modified by a poor venepuncture and so the accuracy is limited by skill in making punctures, by the size of the vein and by sharpness and size of the needles.

TABLE I

TIME IN MINUTES

A		B				C
EXP. 44	EXP. 38	EXP. 6	EXP. 36	EXP. 59		EXP. "BLACKIE"
7.75	6.5	4.0*	6.0*	9.0	10.0	9.25
7.75	6.75	7.0	7.0	10.0	10.75	8.75
7.75	6.75	6.75	7.75	7.5*	10.75†	7.0
	7.0		7.75	10.5	10.5	8.0
(Rabbit:				10.25	10.0	8.0
heart puncture)				10.25		7.5

*Poor venepuncture as noted at time of making puncture.
†Venepuncture not very good and trouble with the electric timer.

Column A shows two series of satisfactory checks. Column B three series containing tests which should be discarded because of poor venepunctures and consequent contamination with tissue juices. Column C is a series of supposedly perfect samples taken from the saphenous vein of a whippet. The vein was well distended and samples could be withdrawn with the utmost ease. The explanation of such discordant results may lie in actual changes in the blood or the needle may have scratched the valves of the veins (hardly the opposite wall) and thus introduced contaminating tissue juices. Coagulation time is affected by such factors as food, exercise, excitement, circulation, P_H of the blood, calcium-ion concentration, degree of hydration and other internal factors affecting the constituents of the clotting mechanism. Some of these have been discussed by other workers (Cannon,[3] Mills,[4] Hartman[5]). In any series which varies slightly it seems reasonable to assume that the highest figure represents more nearly the normal or true clotting time. Wide variations however must be considered as actual changes except when it is obvious that the venepuncture was poorly made. Such wide variations must of course be changing consistently in the same direction as in column C,

TABLE II

EXP. 53	
9.5*	9.25
10.25	11.0
10.5	11.5
10.25	9.75*
10.5	11.0

*Rather poor venepuncture.
Last 6 results shown graphically in Fig. 3.

Table I. Table II shows a series (Fig. 3) in which there was apparently a slight increase in the clotting time during the experiment.

SUMMARY

1. A new method of recording graphically the clotting time of blood is described.

2. The method depends on venepuncture or heart puncture (in rabbit) thus minimizing the tissue juice factor.

3. A relatively small quantity of blood is used (1 c.c.).

4. A tracing obtained with the coagulometer gives the "clotting curve."

5. The tracing also indicates the strength of the clot which may be interpreted to be a rough measure of the amount of fibrin in the blood.

6. As the end-point of clotting, a definite point on the clotting curve is taken.

7. Because the clotting curve is in reality a viscosity curve the instrument has been called a "visco-coagulometer."

8. The human factor has been eliminated from all the essential phases of a clotting test except the actual taking of the sample and placing it in the apparatus. This process consumes about twenty seconds. The one uncertain factor is in making the venepuncture.

9. Mechanical and thermal conditions remain identical in every test.

The author wishes to express his appreciation to Dr. A. J. Carlson of the department and Dr. Guy C. Taylor of Flint-Eaton & Co., Decatur, Ill., for helpful suggestions and to Messrs. L. W. Moody, Harry L. Stimson, Norman Cooperman, Lester Martin, and A. W. Morton for their assistance in performing experiments.

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THE USE OF THE INTERFEROMETER FOR SERUM PROTEIN AND PROTEIN FRACTION DETERMINATIONS*

By William J. Dieckmann, B.S., M.D., St. Louis, Mo.

THE interferometer has been used extensively in many European laboratories since the introduction of Zeiss in 1910 of a modification by Löwe of the Rayleigh instrument. Despite the accuracy and ease of manipulation, there are surprisingly few instruments in this country. A full description of the physics involved in interferometry can be found in an article by Beltz and Kaufmann. Adams in 1915 gave a clear and concise description of the interferometer and stated that it was a precision instrument, suitable for all sorts of determinations in which the refractometer was used, with the additional advantage of being much less susceptible to temperature changes and much more accurate. For example, with a refractometer one must regulate the change in temperature to 0.01° in order to secure an accuracy of one unit in the sixth place, but the interferometer requires no special regulation of temperature to secure an accuracy of one unit in the seventh place. This is possible because in the latter case we are comparing the refringence of one liquid (or gas) with that of another of very nearly the same composition and hence possessing almost the same temperature coefficient of refringence. In other words, with the refractometer, one can determine the composition of the solution to two parts in ten thousandths of solvent, but with the interferometer, to two parts in a million. The unknown may be solute or solvent, electrolyte or nonelectrolyte.

The chambers of the water interferometer are supplied in four sizes, 5, 10, 20, and 40 mm. The 5 and 10 mm. chambers can be fitted with a 4 and 9 mm. insert respectively, thus furnishing a chamber 1 mm. in depth with the advantage that it can be easily cleaned on removal of the insert.[1] The chambers can be obtained with either cemented or fused joints, the latter being suitable for strong acid or alkali, or solutions with a strong solvent property The scale is divided into 3,000 divisions or Trommelteil (T.T.), but we limit our readings to 1500 T.T., because it has been found that if the concentration of a solution is plotted against the T.T., the line will no longer be straight above this figure. Therefore, solutions which give readings in one chamber greater than 1500 are read in a smaller chamber or are diluted.

Thus the 1 mm. chamber is approximately three times as accurate, and it is evident that the accuracy increases with the size of the chamber.

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[1]The chambers are cleaned by flushing first with 0.9 per cent NaCl solution, then with water. Each solution in turn is removed by suction. The end of the suction tube is glass, covered with rubber to protect the chamber wall. An air current is used to dry the chamber after flushing.

TABLE I

ILLUSTRATES THE ERROR AND RANGE OF THE INTERFEROMETER

CHAMBER SIZE	ERROR N_D ±	RANGE
1 mm.	0.0000125	1.33320–1.370700
5 mm.	0.0000025	1.33320–1.340100

The error of the refractometer is $n_D = \pm 0.000037$

It has already been mentioned that one advantage of the interferometer is its insensitiveness to temperature changes as compared with ordinary refractometers. The temperature coefficient is not absolutely nil, however; for example, Adams finds that in the case of KCl solutions, it is about –0.2 per cent per degree (at 20-25°), that is, if in reading one KCl solution against another, we find r = 200 T.T. at 25°, the corresponding reading at 20° would be 202. We found that a serum protein at 15° read 454 and at 30°, 452, therefore we have made no attempt to maintain a standard temperature.

We are using the interferometer for many purposes, but wish to describe its use in determining the percentage of serum protein, fibrin, and the albumin-globulin ratio. Beltz and Kaufmann calibrated their interferometer against a refractometer, using the data of Reiss, for protein and nonprotein fraction. Their results with normal sera, having normal nonprotein fractions, are correct, but in cases where the nonprotein portion is high or low, their results likewise will be too high or too low, for they do not determine the nonprotein fraction. Hirsch described a method which basically is similar to that used by Robertson for the refractometer. Our method for serum protein is similar to Robertson's, but differs from Hirsch's in that we do not dilute our sera with water or physiologic salt solution, having found that certain sera become cloudy if diluted and also that the procedure is not only shortened by eliminating the dilution, but made more accurate.

All interferometer chambers have to be standardized against known solutions. Our instrument was standardized in the following way: Serum proteins were precipitated by adding one volume of serum to ten volumes of alcohol. The precipitate was washed three times in the volume of absolute alcohol originally employed for the precipitation and then three times in the same volume of ether. The precipitate was then allowed to settle after each washing and the supernatant fluid drawn off by means of a siphon. The thick suspensions of the protein in ether, which were obtained, were dried over sulphuric acid at room temperature for sample A, four weeks; and B, two years. Sample A was weighed and an exact 4 per cent solution of protein in 0.02N NaOH was made. B was made up in an exact 3 per cent solution of protein in 0.04N NaOH. A was diluted and a 1 per cent solution gave a reading of 150 T.T. The NaOH read 14 T.T.; therefore, the difference, 136 T.T., represents the interference produced by a 1 per cent protein solution. Solution B was diluted with 0.04N NaOH and a 1.5 per cent solution gave a reading of 237 T.T. The 0.04N NaOH read 32 T.T., therefore the difference, 205, represents the interference produced by a 1.5 per cent protein solution or a 1 per cent solution was equal to 136.6 T.T.

Table II represents readings of a standard protein solution which were read in the 1 mm. chamber for the high concentrations and in the 5 mm. chamber for the low. Kjeldahl determinations were also made on different samples, the amounts used varying from 3 to 5 c.c. of the protein solution.

Table III is presented to illustrate the accuracy and sensitivity of the interferometer in comparison with the dipping refractometer. The refractive indexes of the same standard protein solution given in Table II and also of the 0.02N NaOH used to dissolve and dilute the proteins were determined with a Bausch and Lomb dipping refractometer. Two sets of percentages,

TABLE II

PROTEIN PER CENT BY WEIGHT	1 MM. CHAMBER			5 MM. CHAMBER		
	T.T.	DIFFERENCE	PROTEIN PER CENT	T.T.	DIFFERENCE	PROTEIN PER CENT
4.00	553	539	3.96			
2.00	275	261	1.92			
1.00	150	136	1.0	705	641	1.0
0.50	75	61	0.456	373	309	0.483
0.25	45	31	0.235	226	162	0.253
0.125				138	74	0.115
0.0625				108	44	0.068
0.02N NaOH				64		

TABLE III

DIPPING REFRACTOMETER				*REISS	†ROBERTSON	PROTEIN PER CENT		
	READING	N_D	DIFFERENCE			WEIGHT	KJELDAHL	INTERFEROMETER
	63.9	1.34237	0.00879	5.12	4.52	4.00	4.13	3.96
	26.8	1.33773	0.00415	2.41	2.13	2.00	2.01	1.92
	21.3	1.33563	0.00205	1.19	1.05	1.00	0.96	1.00
	18.3	1.33447	0.00089	0.517	0.457	0.50	0.58	0.483
	17.0	1.33397	0.00039	0.227	0.20	0.25		0.253
	16.1	1.33362	0.00004	0.232	0.205	0.125		0.115
0.02N NaOH	16.0	1.33342				0.0625		0.068

*Reiss — n_D 1 per cent protein = 0.00172 and for nonprotein fraction = 0.00277.
†Robertson — n_D 1 per cent protein = 0.00195 and for nonprotein fraction = 0.00234.

Reiss and Robertson, are given. The reason for the difference between these two is evident from the table. Our results indicate that the factor of Robertson for 1 per cent protein will give results closer to the correct ones than that of Reiss. It is clearly evident that the refractometer cannot be used for solution of protein of a concentration less than 0.2 or 0.3 per cent, while the interferometer can measure concentrations of protein of less than 0.1 per cent in the 5 mm. chamber and of still greater dilution in the 10, 20, and 40 mm. chambers.

Serum Protein.—All of our blood specimens are taken without stasis and a portion for certain determinations injected into a centrifuge tube under oil. The clot is broken up in approximately twenty to forty minutes after the venipuncture, the tube centrifuged and the serum separated and kept under oil. Approximately 0.2 c.c. of the serum is placed in the left compartment and pure water in the right compartment, the 4 mm. plate is inserted and when the two solutions are the same temperature as evidenced by all lines being vertical, the drum is turned until the lines match and the T.T.

noted. In a small test tube (usually 75 × 10 mm.) is placed one volume of serum and, with the same pipette, one volume of 0.04N acetic acid[2] (usually 0.5 c.c. of each is used). The tube can either be sealed off or it can be corked and placed in a holder as shown in Fig. 1, and inserted into a boiling water-bath for three minutes. The precipitate is then broken up with a platinum wire, the tube centrifuged and the clear filtrate placed in the left compartment and a reading obtained without the insert. A reading of the acetic acid diluted in a similar manner with water is also obtained. The difference of these readings, multiplied by two, represents the interference produced by the nonprotein constituent of the serum (urea, electrolyte, fats, etc.). This reading is converted by a known factor to the 1 mm. chamber. The difference between this reading and that of the serum represents the coagulable protein. This figure divided by 136, gives the percentage of proteins in the sample taken.

Table IV shows that the reading of filtrate is very exact, irrespective of the amounts of serum precipitated, provided the same pipette is used to measure both acetic acid and serum.

Fig. 1.—The holder is made of brass. A piece of rubber is set in the base to protect the test tube. The tubes are corked with a rubber stopper and placed in the holder. A small, flat, brass piece, 2 mm. thick is placed over the stopper and the screw turned until sufficient pressure is produced to hold the tube in place.

Tables V and VI indicate the need for a filtrate determination with each protein. The former represents the changes produced in a normal individual by the injection of 100 grams of glucose in a period of thirty minutes. If the filtrate A is determined for each sample, the decrease in serum protein due to the dilution can be followed very accurately, but if the filtrates B are assumed to be the same, the protein figures are decidedly different. Table VI represents the changes from day to day of the serum proteins and filtrate in a case of vomiting with a resultant blood concentration, high nonprotein nitrogen, and low chlorides. The need for determining the nonprotein fraction for every protein solution has been emphasized by Robertson, but judging from reports in the literature of protein determinations with the refractometer, the importance of this determination has not been appreciated.

Table VII indicated the close relationship of serum protein percentage as determined by the interferometer in comparison with the Kjeldahl, the latter method requiring at least 5 to 10 times as much serum to obtain the same accuracy, more manipulation with resultant chance for error, more chemicals, and much more time. A single complete determination with the interferometer requires approximately ten minutes, while a single complete

[2]0.04N acetic acid is made by diluting 4 c.c. of glacial acetic acid to 1750 c.c.

Kjeldahl determination, which necessarily includes a determination of the nonprotein nitrogen, requires, at a minimum, one hour.

TABLE IV

	FILTRATE	PROTEIN PER CENT
2.0 c.c. Serum in NaOH + 2.0 c.c. Acetic acid	175	3.0
2.0 c.c. Serum in NaOH + 2.0 c.c. Acetic acid	169	3.01
1.0 c.c. Serum in NaOH + 1.0 c.c. Acetic acid	169	3.01
1.0 c.c. Serum in NaOH + 1.0 c.c. Acetic acid	173	3.0
0.5 c.c. Serum in NaOH + 0.5 c.c. Acetic acid	169	3.01
0.5 c.c. Serum in NaOH + 0.5 c.c. Acetic acid	168	3.02

TABLE V

TIME	T.T. 1 MM.	FILTRATE 1 MM. A	PROTEIN PER CENT		
			INTERFEROMETER A	INTERFEROMETER B	KJELDAHL
0	1145	138	7.40	7.40	7.40
1 min.	1009	169	6.17	6.40	6.12
32 min.	948	190	5.57	5.95	5.40
1 hr. 39 min.	1152	157	7.31	7.45	7.35

A=Filtrate determined for each specimen.
B=Filtrate assumed to be constant, i. e.—138.

TABLE VI

TIME	T.T., 1 MM.	FILTRATE, 1 MM.	PROTEIN PER CENT
3/24/29	1249	243	7.25
3/25/29	1060	246	6.34
3/26/29	936	162	5.67
3/27/29	850	159	5.10
3/28/29	895	150	5.48
3/30/29	980	147	6.10
4/ 1/29	1001	176	6.06
4/ 4/29	1033	144	6.53
4/11/29	963	146	6.00

TABLE VII

SERUM NO.	T.T.	FILT.	PROTEIN PER CENT		
			INTERFEROMETER		KJELDAHL
199	945	162	5.73		5.86
202	997	129	6.36		6.37
209	998	125	6.40		6.37
244	985	152	6.11		6.08
250B	1090	141	6.95		7.05
*100%	871	131	5.43		5.56
				Calculated	
*66.6%	564	84	3.53	3.62	3.50
*50.0%	421	Calculated	2.62	2.72	2.75
*33.3%	273	Calculated	1.70	1.81	1.71
*25.0%	196	Calculated	1.21	1.36	1.36

*Percentage of original serum No. 250, diluted with H_2O.

Fibrin.—Exactly 1 c.c. of citrate plasma is delivered into a beaker containing 28 c.c. of an 0.8 per cent solution of sodium chloride, and 1 c.c. of a 2.5 per cent calcium chloride solution is added. The solution is thoroughly mixed and allowed to stand at room temperature for at least one hour. The fibrin is freed from the plasma elements by gentle manipulation and pressure with a glass rod. The fibrin mass thus obtained is washed in distilled water,

dried with filter paper and dissolved in 2 c.c. of 0.05N NaOH with the aid of heat. The solution is read in the 10 mm. chamber and the difference between the fibrin reading and that of the NaOH divided by 1090 (1 per cent protein in 10 mm. chamber) multiplied by 2, gives the percentage of fibrin in the sample.

Table VIII demonstrates the accuracy with which fibrin determinations can be made with 1 c.c. of plasma. The gravimetric determinations of fibrin were made according to the technic of Foster and Whipple.

TABLE VIII

NUMBER	FIBRIN		
		INTERFEROMETER	
	WEIGHT	T.T.	PER CENT
X	0.460	506	0.464
		504	0.463
		480	0.440
		494	0.450
		522	0.479
		512	0.470
		510	0.468
		522	0.478
		520	0.476
		478	0.440
D	0.250	350	0.321
	0.265	312	0.286
		324	0.297
B	0.450	518	0.474
	0.470	530	0.486
R	0.670	764	0.70
	0.750	806	0.74
		846	0.777
1002		1620	1.49
		1572	1.44
1005		1142	1.05
		1132	1.04
		1124	1.03

Albumin-Globulin Ratio.—Into a 75 × 10 mm. test tube is placed 1 c.c. of serum and 1 c.c. of a 44 per cent solution of Na_2So_4 is added with the same pipette. The tube is corked and shaken, and allowed to stand in the incubator for three hours. It is then filtered in the incubator through a fine, small filter paper. A clear filtrate is obtained which is read against a standard similarly prepared with H_2O and Na_2SO_4, using the same pipette that was used for the serum. Tubes, pipettes, and sera are warmed before use to prevent precipitation of the Na_2SO_4. The reading obtained multiplied by two, is the interference produced by the albumin and nonprotein elements. This figure minus the filtrate gives the albumin and dividing the latter by 136 gives the percentage of serum albumin. The difference between the serum protein and serum albumin is the globulin.

Table IX illustrates that satisfactory results can be obtained for the albumin-globulin ratio.

CONCLUSIONS

The interferometer for many purposes is interchangeable with the refractometer and has the advantage that more accurate results can be obtained with no special regulation of temperature.

The results obtained by the refractometric determination of serum protein are only accurate if the nonprotein filtrate is determined for each serum and the factor of Robertson for 1 per cent protein is used, rather than that of Reiss, which will give results that are too high.

Serum protein, fibrin, and the albumin-globulin ratio can be determined easily, quickly, and accurately on minimum amounts of serum or plasma.

TABLE IX

NUMBER	ALBUMIN		GLOBULIN	RATIO $\frac{A}{G}$
	T.T.	PER CENT		
D	540	3.94	3.27	1.20
	546	3.98	3.23	1.23
	556	4.06	3.15	1.29
E	508	3.70	2.52	1.46
	510	3.73	2.49	1.49
	496	3.62	2.60	1.39
Ch.	474	3.46	2.48	1.39
	450	3.29	2.65	1.24
1622	542	3.95	2.75	1.44
	526	3.84	2.86	1.35
	524	3.83	2.87	1.35
5 c.c. of each	552	4.03	2.67	1.45
5 c.c. of each	540	3.94	2.76	1.43
1624	546	3.98	2.48	1.60
	540	3.95	2.51	1.57

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DETERMINATION OF CHOLESTEROL IN BLOOD PLASMA AND SERUM*

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With the Technical Assistance of Miss Hazel Irving

SO MANY different modifications of Bloor's[1] method for determining cholesterol have been published in recent years that the authors hesitate to increase the number. We believe however that the method reported here has a number of advantages over the others. The chief of these is that the whole determination is carried out without the application of heat; thus reducing to a minimum, if not eliminating, the possibility of the development of a brown color in the unknown, which so often develops when heat is used, and which makes accurate colorimetric readings very difficult. The method is also simple and quite rapid.

Method: Place approximately 2 gm. of Doucil,† previously dried at 120° to 125° C., in a 10 c.c. glass-stoppered graduated cylinder. (The volume of Doucil should be approximately 2.5 to 3.0 c.c.). Add 1 c.c. of chloroform, stopper and shake so as to distribute the chloroform and separate the Doucil particles. Add 0.5 c.c. of plasma or serum directly on the Doucil, stopper and shake again to separate the particles. Add 9 c.c. of chloroform and shake well from one to two minutes. Set the cylinder aside placing it on its side and leaving the Doucil evenly distributed along the tube. Shake occasionally and after an hour filter through a little glass wool.

Transfer 5 c.c. of the filtrate to another 10 c.c. glass-stoppered cylinder. Measure 5 c.c. of a standard solution in chloroform (containing 0.5 mg. of cholesterol) into a similar cylinder. To each of the solutions add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid. Mix by inverting several times, set aside in the dark for fifteen minutes, and compare in a colorimeter. If the day is warm it is advisable to place the cylinders in cold tap water before setting aside in the dark.

$$\text{Calculations} \quad \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 200 = \text{Mg.}$$

cholesterol per 100 c.c. of plasma or serum.

Time required for extraction.—The time required for extraction depends on the efficiency and the duration of the shaking. If the tubes were continuously shaken the time of extraction would undoubtedly be very markedly reduced. An hour is considered sufficient by the method employed.

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†A water softener manufactured by the American Doucil Co., Philadelphia.

TABLE I

CHOLESTEROL CONTENT OF PLASMA AND SERUM EXPRESSED IN MG. PER 100 C.C.

SAMPLE NO.	REMARKS	SACKETT'S METHOD	AUTHORS' METHOD
1	Fresh blood plasma	233	219
2		167	160
3		84	73
4		261	242
5		193	203
6		173	173
7		239	245
8		231	262
	Average	197.6	197.2
9	Pooled Wassermann serum from	106	113
10	1 to 2 days old	168	168
11		186	194
12		146	148
13		140	155
14	Fresh blood serum	177	176
	Average	153.8	159.0

TABLE II

TIME OF EXTRACTION	CHOLESTEROL RECOVERED IN MG. PER 100 C.C.		
	SAMPLE I	SAMPLE II	SAMPLE III
Overnight	---	132	213
	---	---	213
90 min.	213	132	---
60 min.	213	124	---
45 min.	211	132	---
30 min.	142	120	---
20 min.	148	88	---
*About 15 min.	---	---	197

*This tube was shaken for about two and one half minutes and allowed to stand about twelve minutes.

DISCUSSION

The method described is not perfectly satisfactory for whole blood, chiefly due to the fact that the Doucil particles clump together and are difficult to separate, thus making complete extraction very slow. Attempts are being made to modify it so that it may be applicable for whole blood. The presence of a limited amount of water in the Doucil is necessary for complete extraction of the cholesterol in a short time. The amount of water in half a cubic centimeter of plasma or serum is sufficient for this purpose, under the conditions of the experiment.

SUMMARY

A method for the determination of cholesterol in blood plasma and serum has been described. It has been compared with Sackett's modification of Bloor's method and found to give satisfactory results.

The Doucil used in these experiments was kindly supplied by the American Doucil Co., Philadelphia, through the courtesy of Dr. Wm. Stericker. It is also a pleasure to acknowledge a research grant from the Chemical Foundation, which enabled the author to devote most of his time to research.

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2. Sackett, G. E.: J. Biol. Chem. 64: 203, 1925.

A NEW AUTOMATIC PIPETTING MACHINE*

By L. E. Drummond, Upper Darby, Pa.

THE Drummond Pipetting Machine was designed in collaboration with Dr. M. F. Barnes, Director of the Division of Laboratories, Pennsylvania Bureau of Animal Industry, Harrisburg, Pa., in the endeavor to produce a machine which would stand up under the strain of continuous service.

Much of the trouble experienced with machines of various types has been due to the inefficiency of the valves. In the Drummond machine the valve

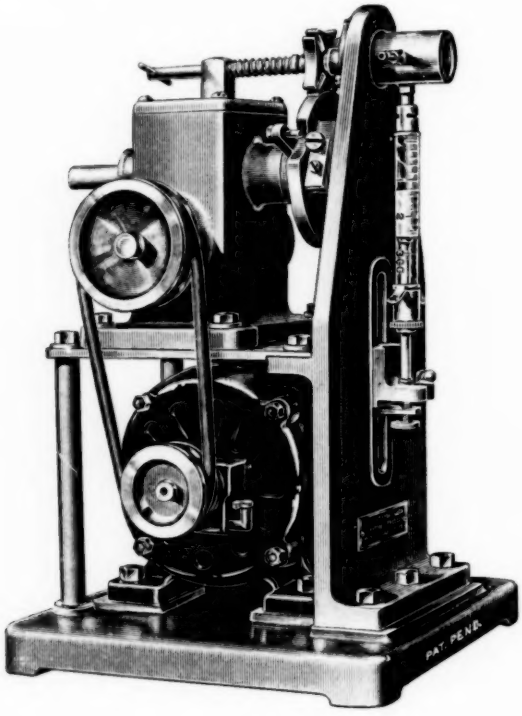

Fig. 1.

mechanism is constructed on the principle of a four-way stopcock which moves forward one quarter turn at each end of the piston stroke. This forward motion of the stopcock plug is accomplished by means of a four-point star wheel actuated by two pins on opposite sides of the driving wheel.

The plug of the stopcock is of stainless steel and the barrel of phosphor bronze. To clean the machine, it is only necessary to lift out the syringe and remove the stopcock plug; the complete cleaning requires but a few moments.

*The Drummond Pipetting Machine is manufactured by Edw. P. Dolbey & Co., 3621 Woodland Ave., Philadelphia, Pa.

The syringe supplied is a Becton Dickinson 3 c.c. Sanalok, which is fitted with the regular locking tip and a monel metal piston. It will be noted that the syringe is mounted tip up in a vertical position; this prevents the accumulation of air therein.

The length of stroke may be adjusted to deliver from 0.1 c.c. to 2.5 c.c. and once set for the desired amount the machine will continue to deliver the same amount at a speed of approximately sixty charges per minute.

The machine is sturdily built and occupies a space of 8″ x 8″. Aside from a sound similar to the ticking of a clock it runs almost silently.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TRICHINOSIS: Human Tr. Spiralis Infections and the Bachman Precipitin Test, Hunter, G. W. Am. J. Hyg. 13: 311, 1931.

In making this, test tubes of a small diameter should be used, such, for example, as might be made by sealing off ordinary glass tubing. At least fifteen or so should be used in each series. An equal amount of serum is added to each tube and the test antigen (Trichinella spiralis protein extract) is added in varying dilutions from 1:500 to 1:200,000 or more. Coca's solution is used as a diluent for the test antigen. This solution may be made up by taking 0.05 gr. $NaHCO_3$, 0.5 gr. NaCl, 0.4 gr. C_6H_5OH in 100 c.c. of distilled water. After the diluted antigen is carefully layered upon the serum, it is necessary to place the test tubes in a water-bath of 37° C. for twenty to thirty minutes, when the tubes should be read, allowed to stand, and reread at the end of thirty minutes and an hour. A clear ring at the interphase of the antigen and serum constitutes a positive test.

The author believes the reaction to be of definite utility.

TISSUE: Staining of Lecithins in Sections, Corria, C. M. R. Arch. Path. 11: 125, 1931.

Frozen sections are placed in acetone, at room temperature, for from one to twenty-four hours. The sections are washed, and are then placed in sudan-acetone, for a few minutes. Then differentiation, washing, mounting, etc., are done. The nuclei stain as in ordinary sudan sections; the acetone soluble lipids are not stained, and the lecithins stain pale red or orange.

TISSUE: Differential Staining of Gram Positive and Gram Negative Bacteria in Tissues. Brown, J. H., and Brenn, L. J. Bacteriol. 21: 21, 1931.

Paraffin sections are prepared as usual for staining.

1. Stain in freshly filtered alum-hematoxylin (Harris) for two to five minutes.
2. Wash in acid alcohol (3 per cent HCl in 95 per cent alcohol) until light pink.
3. Wash in ammonia water (1 c.c. aqua ammoniae in 100 c.c. water) until blue.
4. Wash in water.
5. In a small vial mix 5 drops of 5 per cent aqueous solution of sodium bicarbonate (containing also 0.5 per cent phenol as a preservative) with about 0.75 c.c. of 1 per cent (by weight) aqueous solution of gentian violet. Immediately pour the mixture onto the slide and stain for two minutes.
6. Wash quickly with water.
7. Cover with Lugol's iodine solution for one minute.
8. Wash with water. Blot.
9. Decolorize in 1 part of ether plus 3 parts of acetone, dropping it onto the slide until no more color comes off.
10. Blot.
11. Stain for five minutes with rosanilin hydrochloride (0.005 gm. per 100 c.c. water) or basic fuchsin (0.1 c.c. saturated alcoholic solution per 100 c.c. water).
12. Wash in water. Blot but do not allow the section to dry.
13. Pass through acetone.
14. Decolorize and differentiate by dropping over the section a solution of 0.1 gram picric acid in 100 c.c. acetone until the section becomes a yellowish-pink. This is the most critical stage of the process and should be carried out by holding the slide over a white plate or dish. Most of the fuchsin should be decolorized from the tissue but the gram-negative bacteria should remain red.

WATER: Comparison of the Dominick-Lauter Test for B. Coli in Water With That of "Standard Methods," Leahy, H. W., Freeman, J. W., and Katsampes, C. P. Am. J. Pub. Health 21: 11, 1931.

The corrected formula for the medium follows:

Lactose	10.0 gm.
"Bacto" peptone	10.0 gm.
"Bacto" beef heart	5.0 gm.
$K_2HPO_4.3H_2O$	14.3 gm.
KH_2PO_4	2 gm.

These substances were dissolved in 2 liters of distilled water at a moderate temperature (50 to 60° C.) and when dissolved there were added:

1.6 per cent alcoholic solution of bromocresol purple	4 c.c.
1.0 per cent aqueous solution of erythrosine	4 c.c.
1.0 per cent aqueous solution of methylene blue	20 c.c.

For the 10 c.c. inoculation of suspected water the above medium was tubed in 15 c.c. quantities, but for the smaller amounts 600 c.c. of distilled water were added to a 1 liter batch of this stock and this was tubed in 15 c.c. quantities. For pure culture work 666 c.c. of distilled water should be added to 1 liter of the stock media. The P_H of the medium was determined electrometrically with a quinhydrone electrode and found to be P_H 7.2.

The following résumé is presented:

1. Eleven hundred and sixteen samples of water from 358 different sources have been tested according to the procedure of Standard Methods of Water Analysis, and compared with the new methylene blue bromocresol purple medium of Dominick and Lauter.

2. The per cent confirmation for the total number of tubes showing gas and the total number of samples showing gas was much higher in both cases for the new medium than for standard lactose broth.

3. The per cent confirmation for the standard lactose tubes showing gas in twenty-four hours was higher than the per cent confirmation for the standard lactose broth tubes showing gas in forty-eight hours, but the per cent confirmation with the Dominick-Lauter tubes showing gas in twenty-four hours and forty-eight hours was approximately the same in each case.

4. The per cent confirmation for standard lactose broth increased as the amount of pollution in the water being tested increased, whereas the per cent confirmation for the new medium remained approximately the same.

5. Of the total samples showing a positive presumptive test 100 per cent were confirmed for the new medium whereas only 68.7 per cent were confirmed for standard lactose broth.

6. The new Dominick-Lauter medium was found to be far superior to standard lactose broth for the detection of B. coli in water.

SYPHILIS: Another Modification of the Precipitation Test, Johns, F. M. New Orleans Med. & Surg. J. 83: 5, 1930.

TECHNIC

Antigen (Stock)

Alcoholic extract of powdered beef heart (Difco powdered heart first defatted with two washings in acetone of ten minutes each. Dry thoroughly and extract in incubator for three days with 98 per cent grain alcohol, one gm. powder to 10 c.c. alcohol.)	90.0 c.c.
Alcohol, 98 per cent (grain)	10.0 c.c.
Balsam of Tolu, 10 per cent alcoholic solution (This balsam must be repurified by two precipitations with water from a saturated alcoholic solution.)	4.5 c.c.
Benzoic acid, 1 per cent alcoholic solution	1.25 c.c.
Cholesterin (Pfanstiehl quality)	0.56 gm.

Mix in order given. Solution may be facilitated by incubator temperature.

SERUM TEST

Place 0.15 c.c. of clear serum on a microscopic slide. Spread out over middle third of slide. Allow to dry completely. Electric fan or incubator may be used to advantage. Dilute antigen by placing 0.4 c.c. isotonic saline (0.85 per cent sodium chloride) into a small test tube. With a dry pipette equipped with a rubber bulb take up and add 0.1 c.c. stock antigen to the saline. Mix by aspirating and ejecting the suspension with the pipette. This suspension may be made up in larger quantities and will keep perfectly suspended for the day's use. Add 0.2 c.c. of antigen suspension to the serum; with toothpick spread antigen over serum and by a circular rubbing motion facilitate solution.

Agitate the slide occasionally by rocking it from side to side. The reaction is complete within five minutes. Read by indirect light and discard.

Three readings are possible:

1. Negative, the solution retains a clear opalescence.

2. Definitely positive, a fine, discrete, yellowish granulation forms which is quite definite and should occasion no question after having been once observed.

3. Strongly positive, a heavy, discrete, yellowish granular precipitate which forms very quickly.

Quantitation. A true measure of the reagin, or precipitin, may be obtained in units by fractionating the quantity of serum used, and this is in no way disturbed by artificially altered systems of dilution or "overdosage."

SPINAL FLUID TEST

Place 5 c.c. clear spinal fluid in a conical bottom centrifuge tube.

Add 4 c.c. saturated aqueous solution of ammonium sulphate.

Heat in a water-bath at exactly 56° C. for forty-five minutes.

Centrifuge in electric machine to pack sediment.

Invert tube and drain for at least five to ten minutes. Wipe mouth and inner sides of tube dry.

Add 0.2 c.c. of antigen dilution to sediment in bottom of tube.

Mix, dissolve precipitate and transfer to a microscope slide by means of a capillary pipette.

Read reaction as in the serum test.

DIABETES MELLITUS: Review of 1073 Cases, Wendt, L. F. C., and Peck, F. B. Am. J. Med. Sc. **181:** 52, 1931.

The review covers a period of ten years (1919-1929) and 1073 cases. The Jewish race composed 34.5 per cent and the incidence in males of the whole series was 35.3 and 64.7 per cent for females.

The general results of the survey are tabulated as follows:

A series of 1073 cases of diabetes is presented.

There were twice as many females as males.

The age of obesity was the age of diabetes, of arteriosclerosis, and of gangrene.

Diabetes may develop following excessive nervous and mental strain, and this type is usually mild.

Familial factors were found in 14.8 per cent, and there is a much higher incidence among the more intelligent classes.

Syphilis is probably not as uncommon among diabetics as has been supposed. A case of latent syphilis might possibly become activated under good diabetic management.

Coma and arteriosclerosis are the chief complications. The former may be eradicated by educational measures plus insulin. The latter remains as the toughest problem, and is the cause of the rising death rate from diabetes.

Insulin is necessary for about one-third of diabetics, must be used temporarily for another third, while the remainder can get along without it. Large doses are usually not necessary. Glucose is not necessary in the treatment of most cases of coma.

The cholesterol determination has been of value in following the progress of cases.

A more nearly constant blood-sugar level may be obtained in some patients by bleeding them every eight hours, and less insulin is used by so doing.

The mortality rate is still rising. Arteriosclerosis is now the greatest problem in diabetic management.

RHEUMATOID ARTHRITIS: Etiology of, Cecil, R. L., Nichols, E. E., and Stainsby, W. B. Am. J. Med. Sc. **181:** 12, 1931.

The authors report the results of an intensive bacteriologic study extending over three years.

The cultural method used follows:

For blood cultures 20 c.c. of blood is taken from the arm, placed in two sterile culture tubes, and allowed to clot. The tubes are centrifuged, the serum withdrawn, the clot broken up and transferred to bottles of beef heart infusion broth with a P_H of 7.6. The bottles are placed in the incubator at 37° C. Every seven days subcultures are made from the original flasks on blood-agar plates and in blood-broth tubes. All cultures and transfers are made in a sterile chamber. Joint cultures are made in blood broth by adding a few drops of synovial fluid to each tube of broth. These cultures, like the blood cultures, are incubated for several weeks and subcultured from time to time.

The organisms are thus described:

On culture media the streptococci which have been recovered from the blood and joints of patients with rheumatoid arthritis present a strikingly similar appearance. They all have one feature in common, that is, very slow growth in the original blood-culture flasks. The average time of appearance of these streptococci in the original cultures is approximately fifteen days. Subcultures, on the other hand, grow rapidly on the usual laboratory media. The colony of a typical strain on blood-agar plates presents the appearance of a Streptococcus viridans. On subculture, however, many of these produce some hemolysis both on blood agar and on blood broth. Morphologically the "typical strain" is a small gram-positive coccus, which on solid media grows in short chains. In liquid media longer chains are formed. In blood broth the streptococcus grows readily with the production of a granular or flocculent sediment with a slight turbidity of the media. In plain broth it produces a considerable amount of sediment and throughout the media a heavy granular growth which adheres to the sides of the tube. All of the strains have been insoluble in bile.

The authors believe that a positive agglutination reaction with the "typical strains" is of highly specific and diagnostic value.

Their conclusions follow:

The evidence for the streptococcal origin of rheumatoid arthritis may be summarized as follows:

1. Almost constant presence of streptococci in foci of infection.

2. Streptococci recoverable from blood in 62.3 per cent of rheumatoid patients; 3.9 per cent in pathologic controls; none in healthy controls.

3. Streptococci recoverable from affected joints in 67.3 per cent of rheumatoid patients; from blood or joint in 77 per cent; none from nonrheumatoid joints.

4. High agglutination of "typical strains" of streptococci with serums of 94 per cent of patients with rheumatoid arthritis.

5. Disappearance of agglutinins with recovery from symptoms of arthritis.

6. Biologic identity of streptococci recovered from blood, joint and focus of infection in the same patient, though there have been a number of exceptions to this rule.

7. Reproduction of rheumatoid arthritis in rabbits with "typical strains" of streptococci.

8. Recovery of same streptococci from blood and joints of arthritic rabbits.

9. Striking similarity of histologic changes in rabbits' joints to those in human rheumatoid joints.

THROAT INFECTIONS: Bacteremia in. Ballenger, H. C., Rubin, M. I., and Werner, M. J. A. M. A. **95**: 1828, 1930.

The method used follows:

From 5 to 10 c.c. of blood was drawn from the external jugular or arm veins and placed directly in flasks containing 50 c.c. of nutrient broth. The flasks of broth were heated to approximately 37° C. before being used and were placed in the incubator immediately after the blood was added. The cultures were observed daily for growth over a period of two weeks. Growth was determined by making a gram stain of a loopful of culture. When the broth above the blood remained clear, the culture was not opened but was shaken and replaced in the incubator. The cultures were always streaked on blood agar and examined for growth before being discarded. Later, instead of being placed directly into the mediums, the blood was drawn into sterile flasks containing glass beads and defibrinated blood. Approximately 1 c.c. of the defibrinated blood was placed in each of two tubes of 0.1 per cent dextrose brain broth. Cultures of the remaining blood were made in 50 c.c. of broth as before. In a few cases, aerobic and anaerobic plate cultures were made.

Nose and throat cultures were taken on blood agar, with a few exceptions when broth was used, and the predominating organism was noted.

The study demonstrated that a bacteremia secondary to an acute or a subacute throat infection is not easily demonstrated by bacteriologic examination of the blood stream.

An occasional bacteremia can be shown to accompany an uncomplicated acute throat infection.

DYSENTERY: A Study of B. Dysenteriae, Sonne Type, Koser, S. A., Reiter, D. O., Bortniker, E., and Swingle, E. L. J. Prevent. Med. **4**: 477, 1930.

The Sonne dysentery bacilli appear to constitute a distinct type on the basis of both physiologic characteristics and agglutinative relationships. The 19 Sonne cultures included in the present study possessed the property of fermenting the following sugars with acid production: glucose, lactose (slow), sucrose (slow), usually raffinose (slow), arabinose, rhamnose, levulose, galactose, maltose, trehalose, mannitol, and glycerol. Negative results occurred with xylose, melexitose, dulcitol, sorbitol, erythritol, salicin, and inulin. It is probable that dextrin is only sparingly utilized, if at all. All the Sonne cultures gave a negative indol test, produced no liquefaction of gelatin or digestion of starch and gave no evidence of hydrogen sulphide production in a dextrose-agar-sulphite-ferric-chloride medium. Nitrates were reduced to nitrites.

A number of other dysentery-like organisms, described under a variety of names, were found to be identical with the Sonne type in every respect.

One of the most striking characteristics of the Sonne cultures was the slow acid production from lactose and sucrose, a property shared by certain other miscellaneous cultures, including the dispar type. The two dispar cultures which were studied in this investigation differed from the Sonne strains in their ability to attack xylose and sorbitol and to form indol. They were also distinct serologically. Both types may be readily distinguished from the Flexner organisms.

A review of the recorded isolations of the Sonne type shows that it has been found in many localities in association with dysentery or dysentery-like conditions, particularly of infants.

BLOOD: Red Blood Cell Size in Anemia, Murphy, W. F., and Fitzhugh, G. Arch. Int. Med. **48**: 440, 1930.

The following method was used:

With a dry syringe and needle, 10 c.c. of blood was withdrawn from an arm vein and placed in a test tube previously prepared by evaporating to dryness 2 c.c. of a 1.6 per cent solution of sodium oxalate. After the solution was mixed thoroughly by gently inverting the tube several times, standardized red blood cell-counting pipettes were filled so that the red blood cells might be counted later. A determination of the hemoglobin by the

Sahli method as modified by Osgood and Haskins was made in many instances, although the determinations made by comparison with Tallquist or Dare scales were used occasionally. Smears of the blood were made also so that the diameter of the red blood cells might be measured. In each instance 250 red blood cells were measured in stained smears of blood as described by Bell, Thomas and Means. The oxalated blood was then centrifuged in a straight, thick-walled test tube at high speed (approximately 2,900 revolutions per minute) for one-half hour, and the percentage volume of the cells and plasma was determined after measuring the columns of each.

The average individual cell volume may be determined by dividing the percentage volume of red cells as determined by the hematocrit by the number of red blood cells in 100 c.c. of oxalated venous blood. The figure is recorded in cubic centimeters times ten to the minus eleventh power. This calculation may be carried out according to the following simple formula in which 42 is the percentage volume of red blood cells as determined by the hematocrit and there are 5,000,000 red blood cells per c.mm.

$$\frac{\text{Percentage of cells by hematocrit}}{\text{Number of R.B.C. in 100 c.c. blood}} = \frac{42}{5{,}000{,}000 \times 1{,}000 \times 100} = 8.4 \times 10^{11} \text{ c.c.}$$

The determination of the individual cell volume as suggested by Haden and as above recorded is a more simple means of determining the average cell size than is the measurement of the mean cell diameter.

Determinations of the individual cell volume are very helpful in differentiating pernicious anemia from other anemias in most instances in which difficulty in diagnosis might arise, the outstanding exception being in the rare condition, so-called aleucemic leucemia. During and shortly following acute loss of blood, there is an increase in the average individual cell volume, whereas in anemia resulting from chronic loss of blood, the individual cell volume is generally low.

Figures presented suggest that with adequate liver therapy, the red blood cells of patients with pernicious anemia return to normal volume. An individual cell volume figure greater than normal should indicate inadequate treatment.

Not only is the method for determining the individual cell volume a simple one to use, but the information so obtained is also as reliable as is that obtained by measurement of the mean diameter of the red blood cells by the method used.

In 31 normal cases the average cell volume was 84 cubic microns ($8.4 \times 10 - 11$ c.c.); in 106 cases of pernicious anemia the average was 10×10^{-11} c.c. or greater. In 52 cases of anemia due to chronic loss of blood only about 20 per cent fell within the normal range there being variations from 5.2×10^{-11} c.c. to 8.5×10^{-11} c.c. In anemia due to acute loss of blood the cell volume was greater than normal immediately following the blood loss, but gradually returned to normal.

In secondary anemia not due to loss of blood the cell volume was below normal, the anemia of nephritis showing volumes above the normal, however.

SPUTUM: Review of the Incubation Method of Concentration. Sweany, H. C., and Stadnichenko, A. Am. Rev. Tubere. 22: 420, 1930.

The method used is as follows:

The sputum is collected preferably for twenty-four hours, especially if scanty; the bottles are prepared as previously described and placed in an incubator fifteen to sixteen hours, after which they are removed and uncorked, and an equal quantity of 3 per cent sodium hydroxide is added. The cork is replaced and the bottle put in the shaking machine and shaken for five to ten minutes. The contents are then poured into a clean centrifuge tube and centrifugated ten minutes at 3000 r.p.m. These tubes are carefully inverted and the supernatant liquid entirely poured off, leaving a packed sediment in the bottom with as little liquid as possible. The smears are made from the top of this sediment by means of a platinum-wire spatula, curved to form a scoop. In order to prevent the action of the

sodium hydroxide during its concentration in evaporation on the slide, the scoop is first dipped in 10 per cent HCl. (3 per cent absolute HCl.) to neutralize the slight amount of alkali present. The smears are made as usual, and the staining done according to Cooper's modification of the Ziehl-Neelsen method. Since the earlier publication, Cooper has emphasized the fact that it is better to cool the slides for thirty minutes after staining, to crystallize the dye. This is truly a worth-while procedure, and illustrates the necessity of adhering strictly to prescribed technic.

The following conclusions are drawn:

1. The results of 106,464 sputum examinations by the incubation-concentration method are reported of which 16,103 were positive.

2. Fifteen per cent more positives were obtained by concentration than by direct smear in a control series of 1,500 specimens.

3. No false positives were obtained in 6,283 examinations on negative controls consisting of observation cases, nontuberculous diseases, and closed tuberculosis.

4. Sixteen positive diagnoses were justifiably questioned because they did not conform to the clinical and x-ray findings at the time of the sputum examination.

5. Of the sixteen, 6 were proved to be tuberculous, 6 were probably tuberculous, one was probably not tuberculous, but one in which the bacilli were avirulent or indifferent; while 3 were most probably errors in technic. This virtually leaves four errors in 16,103 positives, or one in about 4,000.

6. Atypical acid-fast forms, when found alone, should be considered only suspicious until proved otherwise.

7. There appear to be no more sources of error with this method than there are with any other method of similar technical manipulation.

ALCOHOLISM: Chemical Proof of Drunkenness and Chemical Measurement of Its Intensity, Novelle, F. Schweiz. Apoth.-Ztg. 67: 246, 1929.

The method depends upon the determination of ethyl alcohol in the blood.

To 10 c.c. of blood in a distilling tube add 65 c.c. of saturated picric acid solution and boil, conducting the vapor into 5 c.c. of water until 20 c.c. of distillate is collected. Place 2 c.c. of distillate in a test tube, add 2 drops of 1.9 per cent potassium bichromate solution and 2 c.c. of concentrated sulphuric acid.

Boil rapidly and then add the potassium bichromate solution from a microburette until the greenish blue color turns slightly green.

1 c.c. of the bichromated solution is reduced by 5 mg. of ethyl alcohol in acid medium.

An alcoholic content of the blood of 0.1 per cent signifies slight, above 0.15 per cent, distinct, 0.3 per cent very severe intoxication, 0.35 to 0.4 per cent resulting in coma or death.

Ether, chloral, salicylic acid, cyanides, chloroform, and other oxidizable substances interfere.

TISSUE STAIN: Modification of MacCallum's Hematoxylin Method for Iron, Dieterle, R. R. Arch. Path. 10: 740, 1920.

Fix tissues in 95 per cent alcohol for five days.

Cut on the microtome after embedding in celloidin or paraffin (brain tissue may be cut unembedded as for Nissl's original method).

Treat the sections for one hour at 55° C. in nitric acid-alcohol: 4 c.c. of nitric acid plus 96 c.c. of 95 per cent alcohol.

Wash twice in distilled water.

Transfer the sections to the hematoxylin solution: 0.5 gm. of hematoxylin, dissolving by heating in 100 c.c. of distilled water, afterward cooling to room temperature, and then adding 1 c.c. of Merck's blue label neutral formaldehyde solution. The resulting solution is kept in a tightly stoppered bottle at 55° C. and immediately before use poured into a Stender dish with a ground-glass cover, to prevent the escape of the formaldehyde. At the

resulting temperature, the staining reaction occurs almost immediately. The sections, however, should remain in the hematoxylin for five minutes. As a control, a section untreated with the nitric acid-alcohol is placed in the solution with the "unmasked" sections. It will be found that this section does not stain. A flocculent precipitate forms within it in a few days; this is removed by filtration. In the course of time another cubic centimeter of formaldehyde can be added to replace the theoretical loss of the same substance. The ion of the nuclei stains an intense bluish black owing to the combining of the hematoxylin with the iron salts formed by the previous acid treatment.

After washing in distilled water, the sections are transferred to equal parts of ether and absolute alcohol for a differentiation, which removes the excess yellow within the tissue.

Follow with neutral xylene and mount in neutral balsam. The preparations are permanent.

The method is useful for the study of the iron content of nuclei. Its application to tumors and to mitoses is especially important. Lately another nuclear stain may be superimposed in the form of a 1 per cent aqueous solution of safranin O (Grubler) for the purposes of studying "acid-nuclei" according to Unna, and have found striking "metachromatic" variations of color in cells of different histologic entity and function. Thus, after differentiation in alcohol and ether, the sections are washed in distilled water, stained with the safranin for twenty minutes, rinsed and differentiated in alcohol made acid by the addition of 10 drops of saturated solution of trinitrophenal or of 3 drops of pure hydrochloric acid in 100 c.c. of 95 per cent alcohol. The sections are then washed in tap water, dehydrated quickly with alcohol, cleared in xylene and mounted in neutral balsam.

Hematoxylin thus demonstrates small traces of inorganic iron, and is more sensitive than the prussian blue or ammonium sulphide methods. For purposes of comparison, the iron of nuclei after unmasking with acid may be treated with the usual potassium ferrocyanide or the ammonium sulphide-potassium ferrocyanide procedures. The blue colored microchemical reactions thus obtained are too faint for accurate comparative study, and possess physical characteristics less suited to ordinary vision and to photomicrography.

ALCOHOL: Determination of in Blood, Hiramatsu, T. Sei-i-Kivai M. J. **48**: 21, 1930.

The principle of the method is based on the fact that the intensity of the black color produced by the reaction between osmium oxide and alcohol is proportional to the concentration of alcohol. To 0.2 c.c. of the test solution, 2 drops of 1 per cent osmium oxide is added and the color developed is determined by matching with standard color tubes. The time required to develop the maximum color depends upon the concentration of alcohol. Over thirty hours or more will be required for a concentration as dilute as 0.02 per cent; this is about the minimum concentration one can determine with 0.2 c.c. sample. With blood, the alcohol should be distilled to be free from the fat and also to be more concentrated. Unlike other methods of determination of alcohol, acetone bodies need not be removed, as such an amount as contained in the blood of severe cases of ketosis will not interfere with this method. Other alcohols beside ethyl alcohol, however, will give a similar color.

KAHN TEST: Reading of in Tinted or Cloudy Sera, Levine, B. S. U. S. Vet. Bur. Med. Bull. **6**: 867, 1930.

Place 1 c.c. of the serum in a tall serologic tube, and make a wax pencil mark to indicate the meniscus of the fluid. Add 2 c.c. saline and mix. Add 3 c.c. saturated solution of ammonium sulphate and mix thoroughly. Centrifugalize the tube at 3000 r.p.m. for fifteen minutes. The precipitate must become *well* packed at the bottom of the tube. Drain off the supernatant fluid thoroughly. Dissolve the precipitate in saline, adding it drop by drop and shaking the tube. The total volume must just reach the wax pencil mark previously made. Use this globulin solution to perform the standard Kahn test as if it were original serum.

B. TUBERCULOSIS: Variations of in Clinical Specimens, Stempa, H. Am. Rev. Tuberc. 22: 443, 1930.

Stempa reports and illustrates variations in the tubercle bacillus in sputum which coincide with those described as part of its life cycle as observed in single cell cultures (Kahn: Am. Rev. Tuberc., 21: 185, 1929).

Stempa used the following technic:

Step 1: Make a very thin film.

Step 2: Dry in air.

Step 3: Fix over a flame, but very carefully.

Step 4: Place the slide in a container with Ziehl-Neelson's carbolfuchsin, in a water-bath, or in any pot with water as long as the water is still cold. Heat the water slowly, to 40° C., and keep it so for fifteen minutes. Take away the heat and leave the container in the water to cool for thirty minutes.

Step 5: Place the container with the slides in an ice box for five minutes.

Step 6: Do not wash in water, but decolorize with 3 per cent acid-alcohol until the slide is absolutely colorless.

Step 7: Now wash in water, and dry gently with a blotter.

Step 8: Pour the counterstain, picric acid, over the film for five seconds. (Saturated aqueous solution of picric acid and alcohol, equal parts.)

Step 9: Wash in water, dry and mount in Canada balsam.

FUNGI: Demonstration of in Skin Scrapings and Hair, Cornbleet, T. J. A. M. A. 95: 1743, 1930.

The reagent, which gives very clear pictures, is prepared as follows:

Several drops of water are sufficient to dissolve a relatively large quantity of sodium sulphide crystals. Equal parts of this solution and 95 per cent alcohol are added to each other. A cloudy precipitate forms at once. Then, by adding a sufficient quantity of distilled water drop by drop, this precipitate redissolves and remains in solution. This completes the preparation. The reagent should be kept in a glass-stoppered bottle that is coated with paraffin at its neck. The alcohol content of the formula will remain relatively more constant if care is taken to close the bottle when not in use.

The alcohol serves a useful purpose in not only removing the oil at the surface of the keratin but also that which is between the scale layers. It does this because it is miscible with water. In this respect it is superior to ether, which is prevented from penetrating the interior by the water in the keratin. The alcohol also helps to attain just that refractive index which gives the optimum visibility of organism structure and transparency of medium.

The Method:

If it is not inconvenient, the scales and hair should first be soaked in ether to remove their surface oil. Otherwise they may be placed directly on a glass slide and covered with a cover slip. Several drops of the reagent are then run beneath the cover slip. Almost at once the keratin structures begin to swell and flatten out after they have made contact with the reagent. In a minute or two the cover slip may be gently pressed down to hasten the clearing process. From five to ten minutes is quite sufficient to produce enough clearing for a good examination. With a solution of potassium hydroxide, several hours is often necessary to obtain satisfactory clearing. Through the microscope the field shows up clearly and the vegetative structures are seen easily and distinctly as much thicker strands than are found when potassium hydroxide is used. Likewise there are many more of them apparent than are seen when the fixed alkalis are used. The walls of the fungi are seen as brilliant blue or green borders that enclose delicately reticulated bands of internal structure.

SPIROCHETES: Simple Stain for, Harris, M. B. K. Science 72: 1863, 1930

Harris highly extols the method described by Kliewe (Centralbl. f. Bakt. 76: 232, 1924).

A film of the material to be examined is prepared as usual, air-dried, and fixed by passing several times through a Bunsen flame. It is mordanted with a 0.5 to 1 per cent aqueous solution of potassium permanganate, washed in water, stained with a 2 per cent

aqueous solution of methyl violet and finally washed in water. The time allowed for the action of the mordant is from eight to ten minutes, while the stain is permitted to act for the same period. In staining Treponema pallida it is often desirable to warm the mordant gently on the slide. It is never necessary to warm the stain. For the coarser more easily stained spirochetes a shorter staining time is required, two to three minutes being quite sufficient. The longer period is preferred for the more delicate organisms. With this method the spirochetes are stained bluish-black and the delicate forms stand out clearly. There is a marked contrast on the slide, and the organisms stained by this method have been photographed without difficulty. Good stained specimens have been obtained of various spirochetes, including Treponema pallidum, Spironema novyi, Spironema obermeieri, Spironema duttoni, Leptospira icterohemorrhagiae, Treponemia vincenti, water leptospira and spirochetes from birds.

LEPROSY: Cultivation of an Acid-Fast Bacillus, Wherry, W. B. Phil. J. Sc. 43: 577, 1930.

The following medium was used:

The white of a hen's egg is boiled in 100 c.c. of distilled water containing 3 to 6 per cent glycerin. This is filtered through cotton. About one-half of the yolk of the egg is thoroughly mixed with the filtrate and boiled. This is filtered through gauze in order to allow the finer yolk particles to pass the filter. This is then autoclaved at 20 pounds for twenty minutes. In the following experiments the glycerinized ovomucoid yolk and nutrient agar containing 2 per cent of agar were mixed aseptically in equal proportions. This medium is semisolid.

Multiplication took place when 1 or 2 drops of autoclaved oleic acid and 1 or 2 drops of a 10 per cent dextrose autoclaved in distilled water were added to each cubic centimeter of the glycerinized agar ovomucoid yolk medium.

Streptococcus hemolyticus was found more frequently in the acutely inflamed throats and the green cocci in the subacutely injected throats.

In the various complications that may be due to an acute throat infection, Streptococcus hemolyticus was found in two-thirds of the cases.

TUBERCULOSIS: Phagocytosis in, Westenrijk, U. Ztschr. f. Tuberc., Leipzig 57: 393, 1930.

Ten drops of blood, secured in a Pasteur pipette from the finger, are mixed in a tube with an equal quantity of 1.5 per cent sodium citrate, centrifuged and the sediment, after removal of the excess citrate solution, washed twice with normal saline.

Three drops of the thin leucocyte layer are placed in a small tube and three drops of an emulsion of tubercle bacilli added. The tube is incubated one hour at 38° C., shaken carefully, and a smear made. Fix in methyl alcohol and dry in the incubator. Stain with Ziehl-Neelsen method and counterstain with saturated methylthionine chloride. Count the number of phagocytes which contain tubercle bacilli.

RUSSELL MEDIUM: The Use of Acid Fuchsin in Russell's Triple Sugar Medium, Cummings, G. D. J. Infect. Dis. 47: 359, 1930.

Cummings, commenting upon the variety of formula for the preparation of Andrade indicator, from studies directed toward a standard formula, reports that the addition of sodium hydroxide is unnecessary.

His studies demonstrate that a satisfactory Russell medium may be made by the addition of 1 or 2 per cent aqueous solution of acid fuchsin to the medium in amounts sufficient to secure a final concentration in the medium of 1 per cent.

The use of undecolorized acid fuchsin has the advantages of chemical stability, ease of preparation, clear-cut reactions, and absence of any inhibitory effect.

TUBERCULIN: The Mantaux vs. the Craig Test, Forbes, R. P., and Steinberg, C. L. Am. J. Dis. Child. **40**: 1230, 1930.

The authors extol the Craig method as the most suitable for use, especially in children. The method follows:

The skin of the forearm is cleansed with ether, and a drop of tuberculin is applied. An ordinary solid sewing needle is held almost parallel to the surface of the skin and it is pressed down firmly and rapidly about sixteen times into the drop of tuberculin. The tuberculin is blotted or wiped off, and the test is read in forty-eight hours. Since the needle is held almost parallel to the skin's surface, it is not possible to penetrate the skin deeply. Firm pressure is necessary, with the result that the point of the needle penetrates the superficial layers of skin as the flesh is deeply depressed. Before and during the procedure the child is told that the dark drop of medicine must be "tickled," and he soon discovers that the procedure is not in the least painful.

Positive reactions appear in from twenty-four to forty-eight hours as red papules with a peripheral zone of erythema. The size of the reaction is almost invariably less than that noted with the Mantoux test. The average diameter of the papules is 4.65 mm.

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EDITORIALS

The Piazza Urine Reaction in Tuberculosis

THE importance of recognizing the presence of tuberculosis while the disease is still in the incipient stages has been reflected in the many endeavors which have been made to evolve some biologic reaction the significance of which would be both clear cut and reliable.

One of the latest concerning which favorable reports have been made is the urinary reaction proposed by Piazza.

This reaction, which is based upon the supposition advanced by Mafucci and again by Maragliano, that tuberculosis is a disease producing alteration in the tissues the products of which may be demonstrated in various secretions and excretions, is comparatively simple in technic, depending upon the precipitation in the urine of the tuberculous antigen by means of a specific antigen.

The precipitin serum is prepared by injecting a rabbit with Koch's old tuberculin.

Four injections are given intraperitoneally (2 c.c., 4 c.c., 6 c.c., and 10 c.c.) the animal being bled fifteen days later.

The test itself is performed as follows: 5 c.c. of urine, which is preferably collected under sterile precautions, and which must be clear and limpid, is placed in each of two test tubes.

Upon the urine in one tube 0.5 c.c. of the precipitin serum is stratified, the other tube serving as a control. Both tubes are incubated at 37° C. for twenty-four hours.

The reaction consists of two phases. The first, or precipitant phase, is read immediately after incubation when, if the urine comes from a tuberculous individual, a precipitate is found in the tube containing urine and precipitin serum. This may vary in intensity from a light granular deposit on the walls of the tube to a definite deposit.

The precipitant phase is considered positive when it does not occur in the control tube or when it is greater in the actual test than in the control.

The second, or lytic phase, is simply the application of the biuret test to both tubes and is considered positive only when it occurs in the test and not in the control.

According to Piazza the reaction can only be expected to be positive in the presence of a febrile reaction and proportionate, not to the extent and gravity of the tuberculous process but to the degree of febrile reaction. This conception, which would rob the test of much of its practical importance, is disputed by some of those reporting upon the reaction, Santoli,[1] for example.

Santoli's results with the Piazza test in 74 cases with marked febrile reactions were so consistently positive that he also applied the test to apyretic cases and a few cases of surgical tuberculosis. He encountered 91 per cent (72 out of 79) positive reactions in pulmonary, surgical and lymphatic tuberculosis, including apyretic forms showing only apical sclerosis and in cases with beginning infiltration and without fever. He believes, therefore, that pyrexia is not essential in the production of a positive reaction.

Santoli also makes the important observation that the administration of arsenous or mercurial drugs inhibits the reaction as well as the presence of blood in the urine.

He believes the Piazza reaction to be specific and of clinical value.

Bellavia[2] has utilized the Piazza test in the study of phlyctenular conjunctivitis. Of 57 cases, 46 were positive to the Piazza test, 21 of these showing manifest tuberculous lesions in the lung. Bellavia concludes, therefore, not only that the Piazza reaction is specific, but that phlyctenular conjunctivitis is not essentially a primary tuberculous process but that it occurs where a tuberculous focus already exists.

These reports are of some interest as indicating, perhaps, the advisability of an extended trial of the Piazza reaction but must, nevertheless, be accepted with definite reserve, first because of the small number of cases involved, and second, because the number and extent of control cases is minimal.

In the evaluation of any procedure it is essential to know its relative specificity. To know the reactions obtained in conditions other than that for which the procedure was devised is at least as essential as to know the incidence of positive reactions in the disease subject to investigation.

All that can be said of the Piazza test so far, then, is that it appears to be worthy of more extended and more extensively controlled investigation.

Like all other procedures, however, it can be expected to be influenced to a greater or lesser extent by the inescapable fact that all biologic, physiologic, or pathologic reactions are governed by two factors: the nature of the stimulus applied and the reacting ability of the individual to whom it is applied.

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—*R. A. K.*

Are Hospitals Teaching Institutions?

THE functions of a hospital are many and that which looms largest very naturally depends upon the angle of view. As regards the intern services, the hospital may be looked upon as the crucible wherein the newborn physician, endowed with a heterogeneous and not always well-aligned nor thoroughly digested assortment of facts and theory, may crystallize abstract theory into concrete practice.

For the successful accomplishment of this highly desirable end, essential to the future practice of medicine in an intelligent manner, it is not sufficient merely for the hospital to be of large size and have a large and busy Staff, for while it is beyond cavil that "experience is the best teacher," what is taught and its eventual value are absolutely dependent upon the character of the experience and the manner in which, and the circumstances under which it is acquired.

There is at all times no little emphasis upon what the resident physician owes the hospital but it is only within comparatively recent times that much has been said about what the hospital owes the resident physician. There can be no doubt that not only are they to furnish such variety of clinical material as shall supply clinical experience, but also they must be prepared to teach the resident physician all that may be learned from its observation and study.

Hospitals, therefore, cannot escape the responsibility thus laid upon them merely by overlooking it. But that it is overlooked if not ignored at times could be demonstrated if resident physicians were consulted on the subject.

There are without doubt hospitals whose resident services are not as profitable as they might be or as they could be made; where residents come and go with but little clarification or crystallization of the experience confronting them.

There are undoubtedly hospitals whose boards would think it unbelievable, in view of their modern equipment, that they were not really teaching their resident physicians, and Staffs who would indignantly repel such an accusation in view of the large number of patients passing through their hands.

An extensive service casually or hastily gone through is not as informative as a much smaller one carefully and methodically studied. A hasty trip through the wards with a Chief who scatters directions here and there, orders another x-ray or what not, or simply announces the diagnosis offhand, does not teach a resident physician *why this* was done rather than something else, or just how the diagnosis was made. It may have sprung from accumulated experience, or may be the result of acute observation, but neither one nor the other will add anything to the information of the resident if they remain locked up in the bosom of the Chief.

It is all very well to expect and demand of the resident physician—as it is, indeed, demanded and expected of any doctor worth his salt—that he shall study and read and consult his books, but some of the practice of medicine must be learned at the bedside. The resident physician, too, has just been freed from the thralldom of books and is eager to learn at the bedside, in the wards and the operating room where there is action and color, and events to arouse and stimulate his interest. He may not be eager to have his Chief lecture to him, but he does not want him, on the other hand, to be inarticulate.

He expects him, of course, to be aware of all the latest developments in order that he may learn from "The Chief" just how they measure up on the light of experience.

He expects the Chief to discuss with him the vital features of the cases displayed before them, not necessarily didactically but at least clearly and logically.

A Chief has it in his power not only to teach—which is a duty he may evade or ignore but cannot eliminate—but to mold the doctors of the future. He may turn the resident physician into a cynical, slipshod worker or inspire and stimulate him to eager, honest effort.

The responsibility placed upon hospitals and their Staffs who accept interns for training is great, and such institutions—their boards and Staffs—must face the question:

Are hospitals teaching institutions?

Are *we* teaching?

—*R. A. K.*